OPIC
Office de la propriété intellectuelle du Canada

CIPO
Canadian Intellectual Property Office

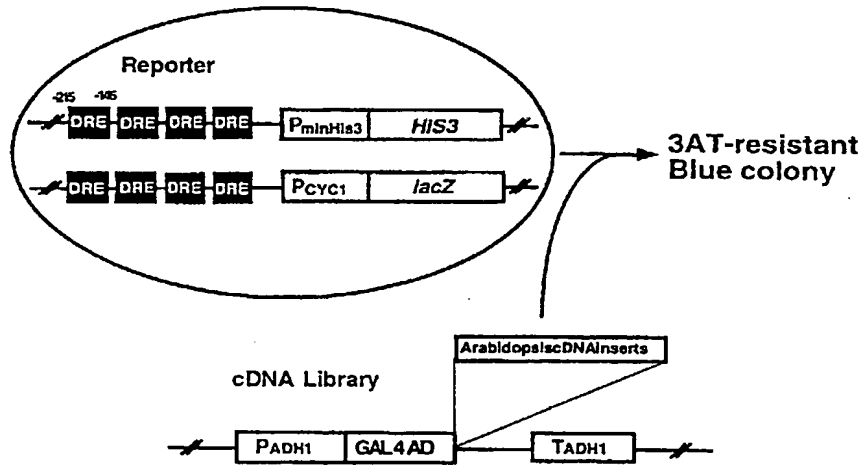
(12) (19) (CA) **Demande-Application**

(21) (A1) **2,269,105**
(22) 1999/04/29
(43) 2000/04/14

(72) SHINOZAKI, Kazuko, JP
(72) KASUGA, Mie, JP
(71) DIRECTOR GENERAL OF JAPAN INTERNATIONAL RESEARCH CENTER FOR AGRICULTURAL SCIENCES, MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES, JP
(71) BIO-ORIENTED TECHNOLOGY RESEARCH ADVANCEMENT INSTITUTION, JP

(51) Int.Cl.[6] A01H 5/00
(30) 1998/10/14 (292348/1998) JP

(54) **PLANTES TOLERANTES AU STRESS ENVIRONNEMENTAL**
(54) **ENVIRONMENTAL STRESS-TOLERANT PLANTS**



(57) The present invention relates to an environmental stress-tolerant plant. The invention discloses a transgenic plant containing a gene in which a DNA encoding the following protein (a) or (b) is ligated downstream of a stress responsive promoter: (a) a protein consisting of the amino acid sequence as shown in SEQ I D NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10; (b) a protein which consists of the amino acid sequence having deletion, substitution or addition of at least one amino acid in the amino acid sequence as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10 and which regulates the transcription of genes located downstream of a stress responsive element.

ABSTRACT

The present invention relates to an environmental stress-tolerant plant. The invention discloses a transgenic plant containing a gene in which a DNA encoding the following protein (a) or (b) is ligated downstream of a stress responsive promoter:

(a) a protein consisting of the amino acid sequence as shown in SEQ I D NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10;

(b) a protein which consists of the amino acid sequence having deletion, substitution or addition of at least one amino acid in the amino acid sequence as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10 and which regulates the transcription of genes located downstream of a stress responsive element.

ENVIRONMENTAL STRESS-TOLERANT PLANTS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a transgenic plant containing a gene in which a DNA encoding a protein that binds to dehydration responsive element (DRE) and regulates the transcription of genes located downstream of DRE is ligated downstream of a stress responsive promoter.

2. Prior Art

In the natural world, plants are living under various environmental stresses such as dehydration, high temperature, low temperature or salt. Unlike animals, plants cannot protect themselves from stresses by moving. Thus, plants have acquired various stress tolerance mechanisms during the courses of their evolution. For example, low temperature tolerant plants (Arabidopsis thaliana, spinach, lettuce, garden pea, barley, beet, etc.) have less unsaturated fatty acid content in their biomembrane lipid than low temperature sensitive plants (maize, rice, pumpkin, cucumber, banana, tomato, etc.). Therefore, even when the former plants are exposed to low temperatures, phase transition is hard to occur in their biomembrane lipid and, thus, low temperature injury does not occur easily.

To date, dehydration, low temperature or salt tolerant lines have been selected and crossed in attempts to artificially create environmental stress tolerant plants. However, a long time is needed for such selection, and the crossing method is only applicable between limited species. Thus, it has been difficult to create a

plant with high environmental stress tolerance.

As biotechnology progressed recently, trials have been made to create dehydration, low temperature or salt tolerant plants by using transgenic technology which introduces into plants a specific, heterologous gene. Those genes which have been used for the creation of environmental stress tolerant plants include synthesis enzyme genes for osmoprotecting substances (mannitol, proline, glycine betaine, etc.) and modification enzyme genes for cell membrane lipid. Specifically, as the mannitol synthesis enzyme gene, Escherichia coli-derived mannitol 1-phosphate dehydrogenase gene [Science 259:508-510 (1993)] was used. As the proline synthesis enzyme gene, bean-derived Δ^1-proline-5-carboxylate synthetase gene [Plant Physiol. 108:1387-1394 (1995)] was used. As the glycine betaine synthesis enzyme gene, bacterium-derived choline dehydrogenase gene [Plant J. 12:1334-1342 (1997)] was used. As the cell membrane lipid modification enzyme gene, Arabidopsis thaliana-derived ω -3 fatty acid desaturase gene [Plant Physiol. 105:601-605 (1994)] and blue-green alga-derived Δ9 desaturase gene [Nature Biotech. 14:1003-1006 (1996) were used. However, the resultant plants into which these genes were introduced were instable in stress tolerance or low in tolerance level; none of them have been put into practical use to date.

Further, it is reported that a plurality of genes are involved in the acquisition of dehydration, low temperature or salt tolerance in plants [Plant Physiol., 115:327-334 (1997)]. Therefore, a gene encoding a transcription factor capable of activating simultaneously the expression of a plurality of genes involved in the acquisition of stress tolerance has been introduced into plants, yielding plants

with high stress tolerance. However, when a gene which induces the expression of a plurality of genes is introduced into a host plant, the genes are activated at the same time. As a result, the energy of the host plant is directed to production of the products of these genes and intracellular metabolism of such gene products, which often brings about delay in the growth of the host plant or dwarfing of the plant.

OBJECTS AND SUMMARY OF THE INVENTION

It is an object of the present invention to provide a transgenic plant containing a gene in which a DNA encoding a protein that binds to a stress responsive element and regulates the transcription of genes located downstream of the element is ligated downstream of a stress responsive promoter, the transgenic plant having improved tolerance to environmental stresses (such as dehydration, low temperature and salt) and being free from dwarfing.

Toward the solution of the above problem, the present inventors have cloned a novel transcription factor gene that regulates the expression of genes involved in the acquisition of dehydration, low temperature or salt stress tolerance, and introduced into a plant this novel gene ligated downstream of a stress responsive promoter. As a result, the inventors have succeeded in creating a plant which has remarkably improved tolerance to dehydration, low temperature or salt and which is free from dwarfing. Thus, the present invention has been achieved.

The present invention relates to a transgenic plant containing a gene in which a DNA encoding the following protein (a) or (b) is ligated downstream of a stress responsive promoter:

(a) a protein consisting of the amino acid sequence as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10;

(b) a protein which consists of the amino acid sequence having deletion, substitution or addition of at least one amino acid in the amino acid sequence as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10 and which regulates the transcription of genes located downstream of a stress responsive element.

Further, the present invention relates to a transgenic plant containing a gene in which the following DNA (c) or (d) is ligated downstream of a stress responsive promoter:

(c) a DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9;

(d) a DNA which hybridizes with the DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9 under stringent conditions and which codes for a protein that regulates the transcription of genes located downstream of a stress responsive element.

Specific examples of the stress include dehydration stress, low temperature stress and salt stress.

As the stress responsive promoter, at least one selected from the group consisting of rd29A gene promoter, rd29B gene promoter, rd17 gene promoter, rd22 gene promoter, DREB1A gene promoter, cor6.6 gene promoter, cor15a gene promoter, erd1 gene promoter and kin1 gene promoter may be given.

This specification includes part or all of the contents as described in the specification and/or drawings of Japanese Patent Application No. 10-292348, which is a priority document of the present application.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a diagram showing the principle of screening of DREB genes.

Fig. 2 shows the structures of probes used in a gel shift assay on the DRE-binding property of DREB1A and DREB2A proteins and presents electrophoresis photographs showing the results of the gel shift assay.

Fig. 3 presents diagrams showing the transcription activating ability of DREB1A and DREB2A proteins.

Fig. 4 is a diagram showing the structure of a CaMV35S promoter-containing recombinant plasmid to be introduced into a plant.

Fig. 5 presents electrophoresis photographs showing transcription levels of individual genes in DREB1A gene-introduced plants when stress is loaded.

Fig. 6 presents photographs showing the growth of DREB1A gene-introduced plants when freezing stress or dehydration stress is given (morphology of organisms).

Fig. 7 is a diagram showing the structure of a rd29A gene promoter-containing recombinant plasmid to be introduced into a plant.

Fig. 8 presents photographs showing the growth of pBI35S:DREB1A-introduced transgenic plants (morphology of organisms).

Fig. 9 presents photographs showing the growth of pBI29AP:DREB1A-introduced transgenic plants (morphology of organisms).

Fig. 10 presents photographs showing the survival of transgenic plants after stress loading (morphology of organisms).

DETAILED DESCRIPTION OF THE INVENTION

Hereinbelow, the present invention will be described in detail.

The transgenic plant of the invention is a environmental stress tolerant, transgenic plant created by introducing a gene in which a DNA (called "DREB gene") encoding a transcription factor that binds to a dehydration responsive element (DRE) and activates the transcription of genes located downstream of DRE is ligated downstream of a stress responsive promoter.

The DREB genes used in the invention can be cloned as described below. Of these DREB genes, DRE-binding protein 1A gene is called DREB1A gene; DRE-binding protein 1B gene is called DREB1B gene; DRE-binding protein 1C gene is called DREB1C gene; DRE-binding protein 2A gene is called DREB2A gene; and DRE-binding protein 2B gene is called DREB2B gene.

1. Cloning of DREB Gene

1-1. Preparation of mRNA and a cDNA Library from Arabidopsis thaliana

As a source of mRNA, a part of the plant of Arabidopsis thaliana such as leaves, stems, roots or flowers, or the plant as a whole may be used. Alternatively, the plant obtained by sowing seeds of Arabidopsis thaliana on a solid medium such as GM medium, MS medium or #3 medium and growing the resultant seedlings aseptically may be used. The mRNA level of DREB1A gene in Arabidopsis thaliana plants increases when they are exposed to low temperature stress (e.g. 10 to -4℃). On the other hand, the mRNA level of DREB2A gene increases when plants are exposed to salt stress (e.g. 150-250 mM NaCl) or dehydration stress (e.g. dehydrated state). Therefore, Arabidopsis thaliana plants which have been exposed to such stress may also be used.

mRNA is prepared, for example, by exposing Arabidopsis thaliana plants grown on GM medium to the dehydration stress, low temperature stress or salt stress mentioned above and then freezing them with liquid nitrogen. Subsequently, conventional techniques for mRNA preparation may be used. For example, the frozen plant are ground in a mortar. From the resultant ground material, a crude RNA fraction is extracted by the glyoxal method, the guanidine thiocyanate-cesium chloride method, the lithium chloride-urea method, the proteinase K-deoxyribonuclease method or the like. From this crude RNA fraction, poly(A)$^+$ RNA (mRNA) can be obtained by the affinity column method using oligo dT-cellulose or poly U-Sepharose carried on Sepharose 2B or by the batch method. The resultant mRNA may further be fractionated by sucrose gradient centrifugation or the like.

Single-stranded cDNA is synthesized using the thus obtained mRNA as a template; this synthesis is performed using a commercial kit (e.g. ZAP-cDNA Synthesis Kit: Stratagene), oligo(dT)$_{20}$ and a reverse transcriptase. Then, double-stranded cDNA is synthesized from the resultant single-stranded cDNA. An appropriate adaptor such as EcoRI-NotI-BamHI adaptor is added to the resultant double-stranded cDNA, which is then ligated downstream of a transcriptional activation domain (such as GAL4 activation domain) in a plasmid (such as pAD-GAL4 plasmid: Stratagene) containing such a domain to thereby prepare a cDNA library.

1-2. A Host to Be Used in the Cloning of DREB Gene

DREB gene can be cloned, for example, by one hybrid screening method using yeast. Screening by this method may be performed using a commercial kit (e.g. Matchmaker One Hybrid System: Clontech).

In the cloning of DREB gene using the above-mentioned kit, first, it is necessary to ligate a DNA fragment comprising DRE sequences to which a protein encoded by DREB gene (i.e. DREB protein) binds to both plasmids pHISi-1 and pLacZi contained in the kit. Then, the resultant plasmids are transformed into the yeast contained in the kit (Saccharomayces cerevisiae YM4271) to thereby prepare a host yeast for cloning.

The host yeast for cloning can biosynthesize histidine by the action of HIS3 protein which is expressed leakily by HIS3 minimum promoter. Thus, usually, this yeast can grow in the absence of histidine. However, since the promoter used for the expression of the gene encoding HIS3 protein is a minimum promoter which can only maintain the minimum transcription level, HIS3 protein produced in cells is extremely small in quantity. Therefore, when the host yeast is cultured in the presence of 3-AT (3-aminotriazole) that is a competitive inhibitor against HIS3 protein, the function of HIS3 protein in cells is inhibited by 3-AT in a concentration dependent manner. When the concentration of 3-AT exceeds a specific level, HIS3 protein in cells becomes unable to function and, as a result, the host yeast becomes unable to grow in the absence of histidine. Similarly, lacZ gene is also located downstream of CYC1 minimum promoter. Thus, β-galactosidase is produced only in extremely small quantity in the yeast cells. Therefore, when the host yeast is plated on an Xgal containing plate, colonies appearing thereon do not have such Xgal degrading ability that turns the colonies into blue as a whole. However, when a transcription factor that binds to DRE sequences located upstream of HIS3 and lacZ genes and activate the transcription thereof is expressed in the host yeast, the yeast

becomes viable in the presence of a sufficient amount of 3-AT and, at the same time, Xgal is degraded to turn the colonies into blue.

As used herein, the term "dehydration responsive element (DRE)" refers to a cis-acting DNA domain consisting of a 9 bp conserved sequence 5'-TACCGACAT-3' located upstream of those genes which are expressed upon exposure to dehydration stress, low temperature stress, etc.

A DNA fragment comprising DRE can be obtained by amplifying the promoter region of rd29A gene (from -215 to -145 based on the translation initiation site of the gene) by polymerase chain reaction (PCR), rd29 gene being one of dehydration tolerance genes [Kazuko Yamaguchi-Shinozaki and Kazuo Shinozaki, The Plant Cell 6:251-264 (1994)]. As a template DNA which can be used in this PCR, genomic DNA from Arabidopsis thaliana is given. As a sense primer, 5'-aagcttaagcttacatcagtttgaaagaaa-3' (SEQ ID NO: 11) may be used. As an antisense primer, 5'-aagcttaagcttgctttttggaactcatgtc-3' (SEQ ID NO: 12) may be used. Other primers may also be used in the present invention.

1-3. Cloning of DREB1A Gene and DREB2A Gene

DREB1A gene and DREB2A gene can be obtained by transforming the cDNA library obtained in subsection 1-1 above into the host obtained in subsection 1-2 above by the lithium acetate method or the like, plating the resultant transformant on LB medium plate or the like containing Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and 3-AT (3-aminotriazole), culturing the transformant, selecting blue colonies appearing on the plate and isolating the plasmids therefrom.

Briefly, a positive clone containing DREB1A gene or DREB2A gene

contains a fusion gene composed of a DNA region coding for GAL4 activation domain (GAL4 AD) and a DNA region coding for a DRE-binding protein, and expresses a fusion protein (hybrid protein) composed of the DRE-binding protein and GAL4 activation domain under the control of alcohol dehydrogenase promoter. Subsequently, the expressed fusion protein binds, through the DRE-binding protein moiety, to DRE located upstream of a reporter gene. Then, GAL4 activation domain activates the transcription of lacZ gene and HIS3 gene. As a result, the positive clone produces remarkable amounts of HIS3 protein and β-galactosidase. Thus, because of the action of the HIS3 protein produced, the positive clone can biosynthesize histidine even in the presence of 3-AT. Therefore, the clone becomes viable in the presence of 3-AT and, at the same time, the Xgal in the medium is degraded by the β-galactosidase produced to turn the colonies into blue.

Subsequently, such blue colonies are subjected to single cell isolation, and the isolated cells are cultured. Then, plasmid DNA is purified from the cultured cells to thereby obtain DREB1A gene or DREB2A gene.

1-4. Homologues to DREB1A Protein or DREB2A Protein

Organisms may have a plurality of genes with similar nucleotide sequences which are considered to have evolved from a single gene. Proteins encoded by such genes are mutually called homologues. They can be cloned from the relevant gene library using as a probe a part of the gene of which the nucleotide sequence has already been known. In the present invention, genes encoding homologues to DREB1A or DREB2A protein can be cloned from the Arabidopsis thaliana cDNA library using DREB1A cDNA or DREB2A cDNA obtained in subsection 1-3

above as a probe.

1-5. Determination of Nucleotide Sequences

The cDNA portion is cut out from the plasmid obtained in subsection 1-3 or 1-4 above using a restriction enzyme and ligated to an appropriate plasmid such as pSK (Stratagene) for sub-cloning. Then, the entire nucleotide sequence is determined. Sequencing can be performed by conventional methods such as the chemical modification method by Maxam-Gilbert or the dideoxynucleotide chain termination method using M13 phage. Usually, sequencing is carried out with an automated DNA sequencer (e.g. Perkin-Elmer Model 373A DNA Sequencer).

SEQ ID NO: 1 shows the nucleotide sequence of DREB1A gene, and SEQ ID NO: 2 the amino acid sequence of the protein encoded by this gene. SEQ ID NO: 3 shows the nucleotide sequence of DREB2A gene, and SEQ ID NO: 4 the amino acid sequence of the protein encoded by this gene. SEQ ID NO: 5 shows the nucleotide sequence of DREB1B gene, and SEQ ID NO: 6 the amino acid sequence of the protein encoded by this gene. SEQ ID NO: 7 shows the nucleotide sequence of DREB1C gene, and SEQ ID NO: 8 the amino acid sequence of the protein encoded by this gene. SEQ ID NO: 9 shows the nucleotide sequence of DREB2B gene, and SEQ ID NO: 10 the amino acid sequence of the protein encoded by this gene. As long as a protein consisting of one of the above-mentioned amino acid sequences has a function to bind to DRE to thereby activate the transcription of genes located downstream of DRE, the amino acid sequence may have mutation (such as deletion, substitution or addition) in at least one amino acid. A mutated gene coding for the protein having such mutated amino acid sequence may also be used

in the present invention.

For example, at least 1 amino acid, preferably 1 to about 20 amino acids, more preferably 1 to 5 amino acids may be deleted in the amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8 or 10; at least 1 amino acid, preferably 1 to about 20 amino acids, more preferably 1 to 5 amino acids may be added to the amino acid sequence shown in SEQ ID NO: 2, 4, 8 or 10; or at least 1 amino acid, preferably 1 to about 160 amino acids, more preferably 1 to 40 amino acids may be substituted with other amino acid(s) in the amino acid sequence shown in SEQ ID NO: 2, 4, 8 or 10. A gene coding for a protein having such mutated amino acid sequence may be used in the present invention as long as the protein has a function to bind to DRE to thereby activate the transcription of genes located downstream of DRE.

Also, a DNA which can hybridize with the above-mentioned gene under stringent conditions may be used in the present invention as long as the protein encoded by the DNA has a function to bind to DRE to thereby activate the transcription of genes located downstream of DRE. The "stringent conditions" means, for example, those conditions in which formamide concentration is 30-50%, preferably 50%, and temperature is 37-50 ℃, preferably 42℃.

A mutated gene may be prepared by known techniques such as the method of Kunkel, the gapped duplex method or variations thereof using a mutation introducing kit [e.g. Mutant-K (Takara) or Mutant-G (Takara)] or using LA PCR in vitro Mutagenesis Series Kit (Takara).

Once the nucleotide sequence of DREB gene has been determined definitely, the gene can be obtained by chemical synthesis, by PCR using the cDNA or genomic DNA of the gene as a template, or by

hybridization with a DNA fragment having the above nucleotide sequence as a probe.

The recombinant vectors containing DREB1A gene and DREB2A gene, respectively, were introduced into E. coli K-12 strain and deposited at the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-Chome, Tsukuba City, Ibaraki, Japan) under accession numbers FERM BP-6654 (E. coli containing DREB1A gene) and FERM BP-6655 (E. coli containing DREB2A gene) on August 11, 1998.

2. Determination of the DRE Binding Ability and Transcription Activating Ability of the Proteins encoded by DREB Genes

2-1. Analysis of the DRE Binding Ability of the Proteins encoded by DREB Genes

The ability of the protein encoded by DREB gene (hereinafter referred to as the "DREB protein") to bind to DRE can be confirmed by performing a gel shift assay [Urao, T. et al., The Plant Cell 5:1529-1539 (1993)] using a fusion protein composed of the above protein and GST. A fusion protein composed of DREB1A protein and GST can be prepared as follows. First, DREB1A gene is ligated downstream of the GST coding region of a plasmid containing GST gene (e.g. pGEX-4T-1 vector: Pharmacia) so that the reading frames of the two genes coincide with each other. The resultant plasmid is transformed into E. coli, which is cultured under conditions that induce synthesis of the fusion protein. The resultant E. coli cells are disrupted by sonication, for example. Cell debris is removed from the disrupted material by centrifugation. Then, the supernatant is purified by

affinity chromatography using a carrier such as glutathione-Sepharose to thereby obtain the fusion protein.

Gel shift assay is a method for examining the interaction between a DNA and a protein. Briefly, a DRE-containing DNA fragment labelled with ^{32}P or the like is mixed with the fusion protein described above and incubated. The resultant mixture is electrophoresed. After drying, the gel is autoradiographed to detect those bands which have migrated behind as a result of the binding of the DNA fragment and the protein. In the present invention, the specific binding of DREB1A or DREB2A protein to the DRE sequence can be confirmed by making it clear that the above-mentioned behind band is not detected when a DNA fragment containing a varied DRE sequence is used.

2-2. Analysis of the Transcription Activating Ability of the Proteins Encoded by DREB Genes

The transcription activating ability of the proteins encoded by DREB genes can be analyzed by a trans-activation experiment using a protoplast system from Arabidopsis thaliana. For example, DREB1A cDNA is ligated to pBI221 plasmid (Clontech) containing CaMV35S promoter to construct an effector plasmid. On the other hand, 3 cassettes of the DRE-containing 71 base DNA region obtained in subsection 1-2 above are connected tandemly to prepare a DNA fragment, which is then ligated upstream of TATA promoter located upstream of β-glucuronidase (GUS) gene in pBI221 plasmid to construct a reporter plasmid. Subsequently, these two plasmids are introduced into protoplasts of Arabidopsis thaliana and then GUS activity is determined. If GUS activity is increased by the simultaneous expression of DREB1A protein, it is understood that DREB1A protein

expressed in the protoplasts is activating the transcription of GUS gene through the DRE sequence.

In the present invention, preparation of protoplasts and introduction of plasmid DNA into the protoplasts may be performed by the method of Abel et al. [Abel, S. et al., Plant J. 5:421-427 (1994)]. In order to minimize experimental errors resulted from the difference in plasmid DNA introduction efficiency by experiment, a plasmid in which luciferase gene is ligated downstream of CaMV35S promoter may be introduced into protoplasts together with the two plasmids described above, and β -glucuronidase activity against luciferase activity may be determined. Then, the determined value may be taken as a value indicating the transcription activating ability. β -glucuronidase activity can be determined by the method of Jefferson et al. [Jefferson, R.A. et al., EMBO J. 83:8447-8451 (1986)]; and luciferase activity can be determined using PicaGene Luciferase Assay Kit (Toyo Ink).

3. Creation of Transgenic Plants

A transgenic plant having tolerance to environmental stresses, in particular, low temperature stress (including freezing stress), dehydration stress and salt stress, can be created by introducing the gene obtained in section 1 above into a host plant using recombinant techniques. As a method for introducing the gene into a host plant, indirect introduction such as the Agrobacterium infection method, or direct introduction such as the particle gun method, polyethylene glycol method, liposome method, microinjection or the like may be used. When the Agrobacterium infection method is used, a transgenic plant can be created by the following procedures.

3-1. Preparation of a Recombinant Vector to be Introduced into a Plant and Transformation of Agrobacterium

A recombinant vector to be introduced into a plant can be prepared by digesting with an appropriate restriction enzyme a DNA comprising DREB1A, DREB1B, DREB1C, DREB2A or DREB2B gene obtained in section 1 above, ligating an appropriate linker to the resultant DNA if necessary, and inserting the DNA into a cloning vector for plant cells. As the cloning vector, a binary vector type plasmid such as pBI2113Not, pBI2113, pBI101, pBI121, pGA482, pGAH, pBIG; or an intermediate vector type plasmid such as pLGV23Neo, pNCAT, pMON200 may be used.

When a binary vector type plasmid is used, the gene of interest is inserted between the border sequences (LB, RB) of the binary vector. The resultant recombinant vector is amplified in E. coli. The amplified recombinant vector is introduced into Agrobacterium tumefaciens C58, LBA4404, EHA101, C58C1Rif^R, EHA105, etc. by freeze-thawing, electroporation or the like. The resultant Agrobacterium tumefaciens is used for the transduction of a plant of interest.

In addition to the method described above, the three-member conjugation method [Nucleic Acids Research, 12:8711 (1984)] may also be used to prepare DREB gene-containing Agrobacterium for use in plant infection. Briefly, an E. coli containing a plasmid comprising the gene of interest, an E. coli containing a helper plasmid (e.g. pRK2013) and an Agrobacterium are mixed and cultured on a medium containing rifampicin and kanamycin. Thus, a zygote Agrobacterium for use in plant infection can be obtained.

Since DREB gene encodes a protein which activates transcription, various genes are activated by the action of the expressed DREB

protein in a DREB gene-introduced plant. This leads to increase in energy consumption and activation of metabolism in the plant. As a result, the growth of the plant itself may be inhibited. As a means to prevent such inhibition, it is considered to ligate a stress responsive promoter upstream of DREB gene so that the DREB gene is expressed only when a stress is loaded. Specific examples of such a promoter include the following ones:

rd29A gene promoter [Yamaguchi-Shinozaki, K. et al., The Plant Cell 6:251-264 (1994)]

rd29B gene promoter [Yamaguchi-Shinozaki, K. et al., The Plant Cell 6:251-264 (1994)]

rd17 gene promoter [Iwasaki, T. et al., Plant Physiol., 115:1287 (1997)]

rd22 gene promoter [Iwasaki, T. et al., Mol. Gen. Genet., 247:391-398 (1995)]

DREB1A gene promoter [Shinwari, Z.K. et al., Biochem. Biophys. Res. Com. 250:161-170 (1988)]

cor6.6 gene promoter [Wang, H. et al., Plant Mol. Biol. 28:619-634 (1995)]

cor15a gene promoter [Baker, S.S. et al., Plant Mol. Biol. 24:701-713 (1994)]

erd1 gene promoter [Nakashima K. et al., Plant J. 12:851-861 (1997)]

kin1 gene promoter [Wang, H. et al., Plant Mol. Biol. 28:605-617 (1995)]

Other promoter may also be used as long as it is known to be stress responsive and to function in plant. These promoters can be

obtained by PCR amplification using primers designed based on a DNA comprising the promoter and using relevant genomic DNA as a template.

If necessary, it is also possible to ligate a terminator which demands termination of transcription downstream of DREB gene. As the terminator, cauliflower mosaic virus-derived terminator or nopaline synthase gene terminater may be used. Other terminator may also be used as long as it is known to function in plant.

If necessary, an intron sequence which enhances the expression of a gene may be located between the promoter sequence and DREB gene. For example, the intron from maize alcohol dehydrogenase (Adh1) [Genes & Development 1:1183-1200 (1987)] may be introduced.

In order to select transformed cells of interest efficiently, it is preferable to use an effective selection marker gene in combination with DREB gene. As the selection marker, one or more genes selected from kanamycin resistance gene (NPTII), hygromycin phosphotransferase gene (htp) which confers resistance to the antibiotic hygromycin on plants, phosphinothricin acetyl transferase gene (bar) which confers resistance to bialaphos and the like.

DREB gene and the selection marker gene may be incorporated together into a single vector. Alternatively, the two genes may be incorporated into separate vectors to prepare two recombinant DNAs.

3-2. Introduction of DREB Gene into a Host Plant

In the present invention, the term "host plant" means any of the following: cultured plant cells, the entire plant of a cultured plant, plant organs (such as leaves, petals, stems, roots, rhizomes, seeds), or plant tissues (such as epidermis, phloem, parenchyma, xylem, vascular bundle). Specific examples of plants which may be used as a

host include Arabidopsis thaliana, tobacco, rice and maize.

DREB gene can be introduced into the above-described host plant by introducing a DREB gene-containing vector into plant sections by the Agrobacterium infection method, particle gun method or polyethylene glycol method. Alternatively, a DREB gene-containing vector may be introduced to protoplasts by electroporation.

If a gene of interest is introduced by the Agrobacterium infection method, a step of infecting a host plant with an Agrobacterium containing a plasmid comprising the gene of interest is necessary. This step can be performed by the vacuum infiltration method [CR Acad. Sci. Paris, Life Science, 316:1194 (1993)]. Briefly, Arabidopsis thaliana is grown in a soil composed of vermiculite and perlite (50:50). The resultant plant is dipped directly in a culture fluid of an Agrobacterium containing a plasmid comprising DREB gene, placed in a desiccator and then sucked with a vacuum pump to 65-70 mmHg. Then, the plant was allowed to stand at room temperature for 5-10 min. The plant pot is transferred to a tray and covered with a wrap to maintain the humidity. The next day, the wrap is removed. The plant is grown in that state to harvest seeds.

Subsequently, in order to select those individuals which have the gene of interest, seeds from various plant bodies are sown on MS agar medium supplemented with appropriate antibiotics. Arabidopsis thaliana grown on this medium are transferred to pots and grown there. As a result, seeds of a transgenic plant into which DREB gene is introduced can be obtained.

Generally, a transgene is located on the genome of the host plant. However, due to the difference in the locations on the genome, the expression of the transgene varies among transformants, presenting a

phenomenon called position effect. Those transformants in which the transgene is expressed more highly can be selected by assaying mRNA levels in transformants by Northern blot analysis using a DNA fragment from the transgene as a probe.

The confirmation that the gene of interest is integrated in the transgenic plant of the invention and in the subsequent generation thereof can be made by extracting DNA from cells and tissues of those plants by conventional methods and detecting the transgene by PCR or Southern analysis known in the art.

3-3. Analysis of Expression Levels and Expression Sites of DREB Gene in Plant Tissues

Expression levels and expression sites of DREB gene in a transgenic plant into which the gene is introduced can be analysed by extracting RNA from cells and tissues of the plant by conventional methods and detecting the mRNA of DREB gene by RT-PCR or Northern blot analysis known in the art. Alternatively, DREB protein may be analysed directly by Western blotting or the like using an antibody raised against the protein.

3-4. Changes in mRNA Levels of Various Genes in a Transgenic Plant in to which DREB Gene is Introduced

It is possible to identify by Northern blot analysis those genes whose expression levels are believed to have been changed as a result of the action of DREB protein in a transgenic plant into which DREB gene is introduced. Northern blotting can assay those genes by comparing their mRNA levels in the transgenic plant into which DREB gene is introduced and in plants into which the gene is not

introduced.

For example, plants grown on GM agar medium or the like are given dehydration and/or low temperature stress for a specific period of time (e.g. 1 to 2 weeks). Dehydration stress may be given by pulling out the plant from the agar medium and drying it on a filter paper for 10 min to 24 hr. Low temperature stress may be given by retaining the plant at 15 to -4 ℃ for 10 min to 24 hr. Total RNA is prepared from control plants which did not receive any stress and plants which received dehydration and low temperature stresses. The resultant total RNA is subjected to electrophoresis. Then, genes expressing are assayed by Northern blot analysis or RT-PCR.

3-5. Evaluation of the Tolerance to Environmental Stresses of the Tra nsgenic Plant

The tolerance to environmental stresses of the transgenic plant into which DREB gene is introduced can be evaluated by setting the plant in a pot containing a soil comprising vermiculite, perlite and the like exposing the plant to various stresses such as dehydration, low temperature and freezing, and examining the survival of the plant. For example, tolerance to dehydration stress can be evaluated by leaving the plant without giving water for 2 to 4 weeks and then examining the survival. Tolerance to freezing stress can be evaluated by leaving the plant at -6 to -10 ℃ for 5 to 10 days, growing it at 20 to 25 ℃ for 5 to 10 days and then examining its survival ratio.

PREFERRED EMBODIMENTS OF THE INVENTION

Hereinbelow, the present invention will be described more

specifically with reference to the following Examples. However, the technical scope of the present invention is not limited to these Examples.

EXAMPLE 1

Cloning of DREB1A Gene and DREB2A Gene

(1) Cultivation of Arabidopsis thaliana Plant

Arabidopsis thaliana seeds obtained from LEHLE SEEDS were sterilized in a solution containing 1% sodium hypochlorite and 0.02% Triton X-100 for 15 min. After rinsing with sterilized water, 40-120 seeds were sown on GM agar medium [4.6 g/L mixed salts for Murashige-Skoog medium (Nihon Pharmaceutical Co., Ltd.), 0.5 g/L MES, 30 g/L sucrose, 8 g/L agar, pH 5.7] and cultured at 22 ℃ under conditions of 16 hr light (about 1000 lux) 8 hr dark, to thereby obtain plant.

(2) Preparation of Poly(A)$^+$ RNA

The plant bodies obtained in (1) above were subjected to low temperature treatment at 4 ℃ for 24 hr, and then total RNA was prepared from them by the glyoxal method. Briefly, 3 g of Arabidopsis thaliana plant frozen in liquid nitrogen was suspended in 100 ml of 5.5 M GTC solution (5.5 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sodium N-lauroyl sarcosinate) and solubilized quickly with a homogenizer. This homogenate was sucked into and extruded from a syringe provided with a 18-G needle repeatedly more than 10 times to thereby disrupt the DNA. Then, the homogenate was centrifuged at 4℃ at 12,000xg for 15 min to precipitate and remove the cell debris.

The resultant supernatant was overlayered on 17 ml of CsTFA solution [a solution obtained by mixing cesium trifluoroacetate

(Pharmacia), 0.25 M EDTA and sterilized water to give D=1.51] placed in an autoclaved centrifuge tube, and then ultracentrifuged in Beckmann SW28 Rotor at 15℃ at 25,000 rpm for 24 hr to precipitate RNA.

The resultant RNA was dissolved in 600 μl of 4 M GTC solution (obtained by diluting the above-described 5.5 M GTC solution with sterilized water to give a GTC concentration of 4 M) and precipitated with ethanol to thereby obtain total RNA of interest.

The resultant total RNA was dissolved in 2 ml of TE/NaCl solution (1:1 mixture of TE and 1 M NaCl) and passed through an oligo-dT cellulose column [prepared by packing a Bio-Rad Econocolumn (0.6 cm in diameter) with oligo-dT cellulose (type 3) (Collaborative Research) to a height of 1.5 cm] equilibrated with TE/NaCl in advance. The solution passed through the column was fed to the column again. Subsequently, the column was washed with about 8 ml of TE/NaCl. TE was added thereto to elute and purify poly(A)$^+$ RNA. The amount of the thus obtained RNA was determined with a UV spectroscope.

(3) Synthesis of a cDNA Library

Double-stranded cDNA was synthesized with a cDNA synthesis kit (Stratagene) using 5 μg of the poly(A)$^+$ RNA obtained in (2) above. Then, the double-stranded cDNA was ligated to pAD-GAL4 plasmid (Stratagene) to thereby synthesize a cDNA library. Briefly, at first, single-stranded cDNA was synthesized in the following reaction solution according to the protocol attached to the kit.

Poly(A)$^+$ RNA	5 μl (5 μg)
10x 1st Strand synthesis buffer	5 μl
DEPC-treated water	34 μl
40 U/μl Ribonuclease inhibitor	1 μl
Nucleotide mix for 1st strand	3 μl
1.4 μg/μl Linker primer	2 μl
Total	50 μl

To the above solution, 1.5 μl (50 U/μl) of reverse transcriptase was added and incubated at 37 ℃ for 1 hr to thereby synthesize single-stranded cDNA. To the resultant reaction solution containing single-stranded cDNA, the following reagents were added in the indicated order.

Reaction solution containing single-stranded cDNA	45 μl
10x 2nd Strand synthesis buffer	20 μl
NTP mix for 2nd strand	6 μl
1.5 U/μl RNase H	2μl
9 U/μl DNA polymerase I	11μl
DEPC-treated water	116 μl
Total	200 μl

The resultant reaction solution was incubated at 16℃ for 2.5 hr to thereby synthesize double-stranded cDNA.

The resultant double-stranded cDNA was blunt-ended by incubating it with 5 units of Pfu DNA polymerase at 72 ℃ for 30 min. Subsequently, the resultant cDNA was subjected to phenol/chloroform

extraction and ethanol precipitation. To the resultant pellet, 9 μl of EcoRI-NotI-BamHI adaptor (Takara), 1 μl of 10x ligase buffer, 1 μl of ATP and 1 μl of T4 DNA ligase (4 U/μl) were added and incubated at 4℃ for 2 days to thereby add the adaptor to the double-stranded cDNA.

Subsequently, the cDNA having an EcoRI restriction enzyme site at both ends was ligated to the EcoRI site downstream of the GAL4 activation domain of pAD-GAL4 plasmid (Stratagene) (a cloning vector) with T4 DNA ligase to thereby synthesize a cDNA library.

(4) Preparation of Genomic DNA

Genomic DNA was prepared from the plant obtained in (1) above according to the method described by Maniatis, T. et al. [Molecular Cloning: A Laboratory Manual, pp. 187-198, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1982)]. Briefly, 2,000 ml of disruption buffer [0.35 M sucrose, 1 M Tris-HCl (pH 8.0), 5 mM $MgCl_2$, 50 mM KCl] was added to 50 g of Arabidopsis thaliana plant. The mixture was disrupted in a whirling blender for 1 min 3 times to homogenize the plant bodies.

The disrupted material was filtered to remove the cell residue. The filtrate was dispersed into centrifuge tubes and centrifuged in a swing rotor at 3,000xg at 4 ℃ for 10 min at a low speed. The resultant supernatant was discarded. The precipitate was suspended in 30 ml of ice-cooled disruption buffer and then re-centrifuged at a low speed. The same procedures were repeated 3 times until the green precipitate turned into white.

The resultant white precipitate was suspended in 10 ml of ice-cooled TE. To this suspension, 10 ml of lysis solution (0.2 M Tris-

HCl (pH 8.0), 50 mM EDTA, 2% sodium N-lauroyl sarcosinate) was added. Then, 0.1 ml of proteinase K (10 mg/ml) was added thereto to digest nuclei. The resultant digest was subjected to phenol treatment and ethanol precipitation. The DNA fiber obtained by the precipitation was recovered by centrifugation at 3,000xg for 5 min and dissolved in 1 ml of TE to thereby obtain genomic DNA.

(5) Construction of a Host Yeast for Use in Yeast One Hybrid Screening

For the cloning of a gene encoding the transcription factor (DRE-binding protein) to be used in the invention, a host was constructed (Fig. 1). This host for cloning comprises two plasmids, one containing 4 cassettes of DRE motif-containing DNA upstream of HIS3 reporter gene and the other containing 4 cassettes of DRE motif-containing DNA upstream of lacZ reporter gene. Briefly, first, the promoter region of rd29A gene (the region from -215 to -145 based on the translation initiation point of rd29A gene) comprising DRE sequence to which the transcription factor to be used in the invention binds to was amplified by PCR. As a sense primer, 5'-aagcttaagcttacatcagtttgaaagaaa-3' (SEQ ID NO: 11) was synthesized. As an antisense primer, 5'-aagcttaagcttgctttttggaactcatgtc-3' (SEQ ID NO: 12) was synthesized. To these primers, a HindIII restriction site was introduced to their 5'end so that PCR fragments can be ligated to a vector easily after amplification. These primers were synthesized chemically with a fully automated DNA synthesizer (Perkin-Elmer). A PCR was performed using these primers and the genomic DNA from (4) above as a template. The composition of the PCR reaction solution was as follows.

Genomic DNA solution	5 μl (100 ng)
Sterilized water	37 μl
10x PCR buffer [1.2 M Tris-HCl (pH 8.0), 100 mM KCl, 60 mM $(NH_4)_2SO_4$, 1% Triton X-100, 0.1 mg/ml BSA]	5 μl
50 pmol/μl Sense primer	1μl (50 pmol)
50 pmol/μl Antisense primer	1μl (50 pmol)
KOD DNA polymerase (KOD-101, TOYOBO)	1μl (2.5 U)
Total	50 μl

After the above reaction solution was mixed thoroughly, 50 μl of mineral oil was overlayered on it. The PCR was performed 25 cycles, one cycle consisting of thermal denaturation at 98℃ for 15 sec, annealing at 65℃ for 2 sec and extension at 74℃ for 30 sec. After completion of the reaction, 50 μl of chloroform was added to the reaction solution, and then the resultant mixture was centrifuged at 4 ℃ at 15,000 rpm for 15 min. The resultant upper layer was recovered into a fresh microtube, to which 100 μl of ethanol was added and mixed well. The mixture was centrifuged at 4℃ at 15,000 rpm for 15 min to pellet the PCR product.

The resultant PCR product was digested with HindIII and then ligated to the HindIII site of vector pSK to yield a recombinant plasmid. This plasmid was transformed into *E. coli*. From the transformant, plasmid DNA was prepared to determine the nucleotide sequence. By these procedures, a transformant comprising pSK with a DNA fragment containing 4 cassettes of DRE connected in the same direction was selected.

The DNA fragment containing 4 cassettes of DRE was cut out from pSK plasmid using EcoRI and HincII, and then ligated to the EcoRI-MluI site upstream of the HIS3 minimum promoter of a yeast expression vector pHISi-1 (Clontech). Likewise, the DRE-containing DNA fragment was cut out from pSK plasmid using EcoRI and HincII, and then ligated to the EcoRI-SalI site upstream of the lacZ minimum promoter of a yeast expression vector pLacZi (Clontech). The resultant two plasmids were transformed into Saccharomyces cerevisiae YM4271 (MATa, ura3-52, his3-200, ade2-101, lys2-801, leu2-3, 112, trp1-903) (Clontech) to thereby yield a host yeast to be used in yeast one hybrid screening (Fig. 1).

(6) Cloning of DREB1A Gene and DREB2A Gene

The host yeast prepared in (5) above was transformed with the cDNA library prepared in (3) above. The resultant yeast transformants (1.2×10^6) were cultured and screened as described previously. As a result, two positive clones were obtained. The cDNAs of these clones were cut out from pAD-GAL4 plasmid using EcoRI and then ligated to the EcoRI site of pSK plasmid to thereby obtain recombinant plasmids pSKDREB1A and pSKDREB2A.

(7) Determination of the Nucleotide Sequences

The entire nucleotide sequences for the cDNAs were determined using plasmids pSKDREB1A and pSKDREB2A. These plasmids were prepared with an automated plasmid preparation apparatus Model PI-100 (Kurabo). For the sequencing reaction, a reaction robot CATALYST 800 (Perkin Elmer) was used. For the DNA sequencing, Perkin Elmer Sequencer Model

373A was used. As a result, it was found that the cDNA from plasmid pSKDREB1A consists of 933 bp (SEQ ID NO: 1) and that only one open reading frame exists therein which encodes a protein consisting of 216 amino acid residues with a presumed molecular weight of about 24.2 kDa (SEQ ID NO: 2). On the other hand, it was found that the cDNA from plasmid pSKDREB2A consists of 1437 bp (SEQ ID NO: 3) and that only one open reading frame exists therein which encodes a protein consisting of 335 amino acid residues with a presumed molecular weight of about 37.7 kDa (SEQ ID NO: 4).

(8) Isolation of Genes Encoding Homologues to DREB1A or DREB2A Protein

Genes encoding homologues to the protein encoded by DREB1A or DREB2A gene obtained in (6) above were isolated. Briefly, genes encoding such homologues were isolated from Arabidopsis thaliana λ gt11 cDNA library using as a probe a double-stranded cDNA fragment comprising DREB1A or DREB2A gene according to the method described by Sambrook, J. et al., Molecular Cloning: A Laboratory Manual 2nd Ed., Cold Spring Harbor Laboratory Press, NY (1989). As genes encoding homologues to DREB1A protein, DREB1B gene and DREB1C gene were obtained; as a gene encoding a homologue to DREB2A protein, DREB2B gene was obtained. As a result of DNA sequencing, it was found that DREB1B gene (SEQ ID NO: 5) was identical with the gene called CBF1 [Stockinger, E.J. et al., Proc. Natl. Acad. Sci. USA 94:1035-1040 (1997)], but DREB1C gene (SEQ ID NO: 7) and DREB2B gene (SEQ ID NO: 9) were found to be novel.

From the analysis of the open reading frame of DREB1C gene, it was found that the gene product encoded by this gene is a protein

consisting of 216 amino acid residues with a molecular weight of about 24.3 kDa (SEQ ID NO: 8). Also, it was found that the gene product encoded by DREB2B gene is a protein consisting of 330 amino acid residues with a molecular weight of about 37.1 kDa (SEQ ID NO: 10).

EXAMPLE 2

Analysis of the DRE-Binding Ability of DREB1A and DREB2A Proteins

The ability of DREB1A and DREB2A proteins to bind to DRE was analyzed by preparing a fusion protein composed of glutathione-S-transferase (GST) and DREB1A or DREB2A protein using E. coli and then performing a gel shift assay. Briefly, the 429 bp DNA fragment from position 119 to position 547 of the nucleotide sequence of DREB1A cDNA or the 500 bp DNA fragment from position 167 to position 666 of the nucleotide sequence of DREB2A cDNA was amplified by PCR. Then, the amplified fragment was ligated to the EcoRI-SalI site of plasmid pGEX-4T-1 (Pharmacia). After the introduction of this plasmid into E. coli JM109, the resultant transformant was cultured in 200 ml of 2x YT medium (Molecular Cloning, (1982) Cold Spring Harbor Laboratory Press). To this culture, 1 mM isopropyl β-D-thiogalactoside which activates the promoter in plasmid pGEX-4T-1 was added to thereby induce the synthesis of a fusion protein of DREB1A (or DREB2A) and GST.

E. coli in which the fusion protein had been induced was suspended in 13 ml of buffer (10 mM Tris-HCl, 0.1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride). Then, 1% Triton X-100 and 1 mM EDTA were added thereto. After the cells were disrupted by sonication, the disrupted material was centrifuged at 22,000xg for 20 min. Then, the

fusion protein of DREB1A (or DREB2A) and GST was purified by affinity chromatography using glutathione-Sepharose (Pharmacia) as a carrier. The resultant fusion protein was incubated with the DRE-containing 71 bp DNA fragment probe prepared by PCR and radiolabelled with ^{32}P at room temperature for 20 min. This mixture was electrophoresed using 6% acryl amide gel containing 0.25xTris-borate-EDTA at 100 V for 2 hr. Fig. 2 shows the results of autoradiogram on the gel after the electrophoresis. As is clear from this Figure, a band which migrated behind was detected when the fusion protein was incubated with the DRE-containing 71 bp DNA fragment probe (SEQ ID NO: 18). When a DNA fragment containing a varied DRE sequence (SEQ ID NO: 19, 20 or 21) was used, such a band was not detected. On the other hand, when a DNA fragment which was partly varied outside of DRE sequence (SEQ ID NO: 22 or 23) was used as a probe, a behind band was detected. Thus, it was shown that DREB1A or DREB2A protein specifically bound to DRE sequence.

EXAMPLE 3

Analysis of the Ability of DREB1A and DREB2A Proteins to Activate the Transcription of Genes Located Downstream of DRE

In order to examine whether DREB1A and DREB2A proteins are able to trans-activate DRE-dependent transcription in plant cells, a trans-activation experiment was conducted using a protoplast system prepared from Arabidopsis thaliana leaves. Briefly, the cDNA of DREB1A or DREB2A was ligated to a pBI221 plasmid containing CaMV35S promoter to thereby construct an effector plasmid. On the other hand, 3 cassettes of the DRE-containing 71 bp DNA region were connected tandemly to prepare a DNA fragment, which was then ligated upstream to

the minimum TATA promoter located upstream of β-glucuronidase (GUS) gene in a plasmid derived from pBI221 plasmid to construct a reporter plasmid. Subsequently, these two plasmids were introduced into protoplasts from Arabidopsis thaliana and then GUS activity was determined. When DREB1A or DREB2A protein was expressed simultaneously, GUS activity increased. This shows that DREB1A and DREB2A proteins are transcription factors which activate transcription through DRE sequence (Fig. 3).

EXAMPLE 4

Creation of a Transgenic Plant Containing a Gene in which a DNA Encoding DREB1A Protein is Ligated Downstream of CaMV35S Promoter

(1) Construction of a Plant Plasmid

Plasmid pSKDREB1A (10 μg) obtained as described above was digested with EcoRV (20 U) and SmaI (20 U) in a buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT) and 100 mM NaCl at 37℃ for 2 hr to thereby obtain a DNA fragment of about 0.9 kb containing DREB1A gene. On the other hand, plasmid pBI2113Not (10 μg) containing promoter DNA was digested with SmaI in a buffer containing 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM DTT and 100 mM NaCl at 37 ℃ for 2 hr. The 0.9 kb DNA fragment containing DREB1A gene and the digested pBI2113Not were treated with T4 DNA ligase (2 U) in a buffer [66 mM Tris-HCl (pH 7.6), 6.6 mM $MgCl_2$, 10 mM DTT, 0.1 mM ATP] at 15 ℃ for 16 hr for ligation. The ligated DNA was transformed into E. coli JM109. The transformant was cultured, and plasmid pBI35S:DREB1A was obtained from the culture. Then, the nucleotide sequence was determined, and those plasmids in which

DREB1A gene was ligated in the sense direction were selected. Plasmid pBI2113Not mentioned above is a plasmid prepared by digesting pBI2113 [Plant Cell Physiology 37:49-59 (1996)] with SmaI and SacI to remove the coding region of GUS gene and ligating a SmaI-NotI-SacI polylinker to the resultant plasmid.

(2) Preparation of a Zygote Agrobacterium Containing the Plant Plasmid pBI35S:DREB1A

E. coli DH5α containing the plant plasmid pBI35S:DREB1A prepared in (1) above, E. coli HB101 containing helper plasmid pRK2013 and Agrobacterium C58 were cultured in mixture on LB agar medium at 28 ℃ for 24 hr. Grown colonies were scraped off and suspended in 1 ml of LB medium. This suspension (10 ml) was plated on LB agar medium containing 100 μg/ml rifampicin and 20 μg/ml kanamycin and cultured at 28 ℃ for 2 days to thereby obtain a zygote Agrobacterium C58 (pBI35S:DREB1A).

(3) Gene Transfer into Arabidopsis thaliana by Agrobacterium Infection

The resultant zygote Agrobacterium was cultured in 10 ml of LB medium containing 100 μg/ml rifampicin and 20 μg/ml kanamycin at 28℃ for 24 hr. Further, this culture fluid was added to 500 ml of LB medium and cultured for another 24 hr. The resultant culture fluid was centrifuged to remove the medium, and the cell pellet was suspended in 250 ml of LB medium.

On the other hand, 4 to 5 Arabidopsis thaliana plant bodies were grown in 9 cm pots containing soil composed of vermiculite and perlite (50:50) for 6 weeks. Then, the plant body was directly dipped

in the LB culture fluid of the Agrobacterium containing plasmid pBI35S:DREB1A and placed in a desiccator, which was sucked with a vacuum pump to reduce the pressure to 650 mmHg and then left for 10 min. Subsequently, the plant pot was transferred to a tray and covered with a wrap to maintain the humidity. The next day, the wrap was removed. Thereafter, the plant was grown uncovered to thereby obtain seeds. After sterilization in an aqueous solution of sodium hypochlorite, the seeds were sown on an agar medium for selection (MS medium supplemented with 100 μ g/ml vancomycin and 30 μ g/ml kanamycin). Arabidopsis thaliana seedlings grown on this medium were transplanted to pots and grown there to obtain seeds of the transformed plant.

(4) Identification of Genes Whose Expression Has Been Altered by the Transgene and the Transcription Factor Encoded by the Transgene

mRNA levels of those genes whose expression is considered to have been altered by the transgene DREB1A and the transcription factor encoded by this gene in the transformed plant were examined by Northern blot analysis. As a probe, a fragment of DREB1A, rd29A, kin1, cor6.6, cor15a, rd17, erd10, P5CS, erd1, rd22 or rd29B gene was used. In this Northern blot analysis, transformed and wild type Arabidopsis thaliana plants were used for comparing the expression of the above genes. Two grams each of plant bodies grown on GM agar medium for 3 weeks were exposed to dehydration stress and low temperature stress separately. Dehydration stress was given by pulling out the plant from the agar medium and drying it on a filter paper for 5 hr. Low temperature stress was given by retaining the plant at 4℃ for 5 hr.

Total RNA was prepared separately from control plants which were given no stress, plants which were given dehydration stress and plants which were given low temperature stress. The resultant total RNA was subjected to electrophoresis. Then, expressing genes were assayed by Northern blot analysis. Generally, a transgene is located on the genome of a transformed plant in a similar manner. However, due to the difference in the locations on the genome, the expression of the transgene varies among transformants; this is a phenomenon called position effect. By assaying transformants by Northern blotting with a DNA fragment from the transgene as a probe, those transformants in which the transgene was expressed more highly were selected. Also, by using as a probe a DNA fragment of the above genes which are possibly involved in stress tolerance, those genes which exhibited changes in mRNA levels when DREB1A gene was introduced were identified (Fig. 5).

(5) Expression of Tolerance to Dehydration/Freezing Stress

Dehydration/freezing tolerance was investigated on Arabidopsis thaliana transformants which had been grown in 9 cm pots containing soil composed of vermiculite and perlite (50:50) for 3 weeks. As a control, Arabidopsis thaliana transformed with pBI121 not containing DREB1A gene was used. As to dehydration tolerance, water supply was stopped for 2 weeks and then plant survival was examined. As to freezing tolerance, the plant was maintained at -6℃ for 2 days and then grown at 22℃ for 5 days. Thereafter, its survival ratio was examined.

As a result, all the control plants were withered but the transgenic plants into which DREB1A gene was introduced exhibited a

high survival ratio (Fig. 6). However, inhibition of growth and dwarfing were observed in these transgenic plants.

EXAMPLE 5

Creation of a Transgenic Plant Containing a Gene in which a DNA Encoding DREB1A Protein is Ligated Downstream of rd29A Gene Promoter

(1) Construction of pBI29APNot Vector Containing rd29A Gene Promoter

An rd29A promoter region (from -861 to +63 based on the translation initiation point of rd29A gene) with HindIII site added to both ends was prepared by PCR under the same conditions as described in (4) in Example 2 using the following primers: 5'-aagcttaagcttgccatagatgcaattcaatc-3' (SEQ ID NO:13) and 5'-aagcttaagcttttccaaagatttttttctttccaa-3' (SEQ ID NO: 14). The resultant PCR fragment was digested with HindIII and inserted into the HindIII site of a plant binary vector pBI101 (Clontech, Palo Alto, CA, USA). β-glucuronidase gene (GUS) encoded in pBI101 was cut out with SmaI and SacI. Then, the resultant plasmid was ligated with SmaI-NotI-SacI polylinker. This plasmid was introduced into E. coli DH5a to prepare plasmid pBI29APNot.

(2) Construction of Plant Plasmid pBI29AP:DREB1A Using rd29A Gene Promoter

DREB1A gene was amplified by PCR using pSKDREB1A obtained in Example 1 as a template. Briefly, 5'-ggatccggatccatgaactcattttctgct-3' (SEQ ID NO: 15) was synthesized as a sense primer and 5'-ggatccggatccttaataactccataacgata-3' (SEQ ID NO: 16) as an

antisense primer. BamHI site was introduced at 5' end of both primers so that the PCR fragment amplified can be ligated to the vector easily. The resultant PCR product was subjected to electrophoresis on 1% agarose gel. A band around 900-1000 bp was cut out from the gel. This gel fragment was placed in a fresh microtube, which was retained at 67 ℃ for 10 min to dissolve the gel. An equal volume of TE was added to the dissolved gel, mixed well and extracted with phenol. The resultant extract was centrifuged at 1,600xg for 3 min. Then, the aqueous layer was subjected to phenol extraction and phenol/chloroform extraction. To the resultant aqueous layer, cold ethanol was added to precipitate the PCR product.

The resultant PCR product (10 μg) was dissolved in 30 μl of TE and digested with BamHI (20 U). After heating at 70℃ for 1 hr to deactivate BamHI, the digest was subjected to phenol extraction and ethanol precipitation to recover a DNA fragment containing DREB1A gene. Subsequently, this DNA fragment was ligated to the BamHI site of vector pBI29APNot. This recombinant plasmid was transformed into E. coli (DH5 α), and the transformant was selected by kanamycin resistance. The selected transformant was cultured in LB medium. Then the plasmid pBI29AP:DREB1A was extracted and purified from the transformant (Fig. 7).

(3) Preparation of a Zygote Agrobacterium Containing Plant Plasmid pBI29AP:DREB1A

Using the recombinant plasmid pBI29AP:DREB1A obtained in (2) above, a zygote Agrobacterium containing plant plasmid pBI29AP:DREB1A was prepared in the same manner as in (2) in Example 5.

(4) Gene Transfer into Arabidopsis thaliana by Agrobacterium Infection

Using the zygote Agrobacterium obtained in (3) above, plant plasmid pBI29AP:DREB1A was introduced into Arabidopsis thaliana in the same manner as in (3) in Example 5.

(5) Observation of the Growth and Dehydration/Freezing/Salt Stress Tolerance of the Transformant

The transgenic Arabidopsis thaliana obtained in (4) above containing a plasmid in which DREB1A gene is ligated downstream of rd29A gene promoter, the transgenic Arabidopsis thaliana obtained in Example 5 containing a plasmid in which DREB1A gene is ligated downstream of CaMV35S gene promoter, and non-transformed Arabidopsis thaliana as a control were cultured under the same conditions. Then, their growth and survival ratios after the loading of dehydration, freezing or salt stress were examined. Briefly, each plant was planted in a 9 cm pot containing soil composed of vermiculite and perlite (50:50) and cultured outside. Figs. 8 and 9 present photographs showing the growth of plants on day 35 (Fig. 8A and Fig. 9A) and on day 65 (Fig. 8B and Fig. 9B) of the cultivation. In the pBI35S:DREB1A-introduced transgenic plant, a remarkable inhibition of growth was observed though there was some difference in the degree of growth among plants (Fig. 8A and Fig. 8B). In contrast, almost no inhibition of growth was observed in the pBI29AP:DREB1A-introduced transgenic plant (Fig. 9A and Fig. 9B).

Subsequently, their tolerance to stresses was examined. As to dehydration tolerance, water supply was stopped for 2 weeks and then plant survival was examined. As to freezing tolerance, plants were

maintained at -6 ℃ for 2 days and then grown at 22℃ for 5 days. Thereafter, their survival ratios were examined. As to salt tolerance, plants were dipped in 600 mM NaCl for 2 hrs, then transferred to pots and grown there for 3 weeks. Thereafter, plant survival was examined. As a result, as shown in Fig. 10 and Tables 1 to 3, the control plants given dehydration or freezing stress were all withered. Only few control plants survived after the loading of salt stress. In the pBI35S:DREB1A-introduced transformant, the survival ratio varied among plants; those plants with higher expression of the introduced DREB1A gene exhibited higher tolerance. In contrast, in the pBI29AP:DREB1A-introduced transformant, the tolerance was almost equal among 43 plants analyzed. This transformant exhibited higher survival ratios than the pBI35S:DREB1A-introduced transformant. Thus, it was found that the transgenic plant created by the invention has high levels of tolerance to dehydration, freezing and salt, and yet exhibits good growth.

Table 1.
Survival Ratio of Transgenic Plants after the Loading of Freezing Stress

	No. of Individuals Survived	Total No. of Individuals	Survival Ratio (%)
rd29A:DREB1A	143	144	99.3
35S:DREB1Ab	47	56	83.9
35S:DREB1Ac	15	42	35.7
Wild type	0	55	0.0

Table 2.
Survival Ratio of Transgenic Plants after the Loading of Dehydration Stress

	No. of Individuals Survived	Total No. of Individuals	Survival Ratio(%)
rd29A:DREB1A	52	80	65.0
35S:DREB1Ab	15	35	42.9
35S:DREB1Ac	6	28	21.4
Wild type	0	25	0.0

Table 3.
Survival Ratio of Transgenic Plants after the Loading of Salt Stress

	No. of Individuals Survived	Total No. of Individuals	Survival Ratio(%)
rd29A:DREB1A	119	149	79.9
35S:DREB1Ab	4	24	16.7
Wild type	4	29	13.8

All publications, patents and patent applications cited herein are incorporated herein by reference in their entirety.

EFFECT OF THE INVENTION

According to the present invention, there is provided a transgenic plant containing a gene in which a DNA coding for a protein that binds to a stress responsive element and regulates the transcription of genes located downstream of the element is ligated downstream of a stress responsive promoter, the transgenic plant having improved tolerance to environmental stresses (such as dehydration, low temperature and salt) and being free from dwarfing.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: DIRECTOR GENERAL OF JAPAN INTERNATIONAL RESEARCH CENTER FOR AGRICULTURAL SCIENCES, MINISTRY OF AGRICULTURE, FORESTRY AND FISHERIES -AND- BIO-ORIENTED TECHNOLOGY RESEARCH ADVANCEMENT INSTITUTION

(ii) TITLE OF INVENTION: ENVIRONMENT STRESS-TOLERANT PLANTS

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: SMART & BIGGAR

(B) STREET: P.O. BOX 2999, STATION D

(C) CITY: OTTAWA

(D) STATE: ONT

(E) COUNTRY: CANADA

(F) ZIP: K1P 5Y6

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: ASCII (text)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: CA 2,269,105

(B) FILING DATE: 29-APR-1999

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP 292348/1998

(B) FILING DATE: 14-OCT-1998

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: SMART & BIGGAR

(B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER: 72813-103

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (613)-232-2486

(B) TELEFAX: (613)-232-8440

(2) INFORMATION FOR SEQ ID NO.: 1:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 933

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE

(A) NAME/KEY: CDS

(B) LOCATION: (119)..(766)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 1:

```
CCTGAACTAG AACAGAAAGA GAGAGAAACT ATTATTTCAG CAAACCATAC CAACAAAAAA 60

GACAGAGATC TTTTAGTTAC CTTATCCAGT TTCTTGAAAC AGAGTACTCT TCTGATCA   118

ATG AAC TCA TTT TCT GCT TTT TCT GAA ATG TTT GGC TCC GAT TAC GAG   166
Met Asn Ser Phe Ser Ala Phe Ser Glu Met Phe Gly Ser Asp Tyr Glu
  1               5                  10                  15

TCT TCG GTT TCC TCA GGC GGT GAT TAT ATT CCG ACG CTT GCG AGC AGC   214
Ser Ser Val Ser Ser Gly Gly Asp Tyr Ile Pro Thr Leu Ala Ser Ser
             20                  25                  30

TGC CCC AAG AAA CCG GCG GGT CGT AAG AAG TTT CGT GAG ACT CGT CAC   262
Cys Pro Lys Lys Pro Ala Gly Arg Lys Lys Phe Arg Glu Thr Arg His
         35                  40                  45

CCA ATA TAC AGA GGA GTT CGT CGG AGA AAC TCC GGT AAG TGG GTT TGT   310
Pro Ile Tyr Arg Gly Val Arg Arg Arg Asn Ser Gly Lys Trp Val Cys
     50                  55                  60

GAG GTT AGA GAA CCA AAC AAG AAA ACA AGG ATT TGG CTC GGA ACA TTT   358
Glu Val Arg Glu Pro Asn Lys Lys Thr Arg Ile Trp Leu Gly Thr Phe
 65                  70                  75                  80

CAA ACC GCT GAG ATG GCA GCT CGA GCT CAC GAC GTT GCC GCT TTA GCC   406
Gln Thr Ala Glu Met Ala Ala Arg Ala His Asp Val Ala Ala Leu Ala
                 85                  90                  95

CTT CGT GGC CGA TCA GCC TGT CTC AAT TTC GCT GAC TCG GCT TGG AGA   454
Leu Arg Gly Arg Ser Ala Cys Leu Asn Phe Ala Asp Ser Ala Trp Arg
            100                 105                 110
```

```
CTC CGA ATC CCG GAA TCA ACT TGC GCT AAG GAC ATC CAA AAG GCG GCG   502
Leu Arg Ile Pro Glu Ser Thr Cys Ala Lys Asp Ile Gln Lys Ala Ala
        115                 120                 125

GCT GAA GCT GCG TTG GCG TTT CAG GAT GAG ATG TGT GAT GCG ACG ACG   550
Ala Glu Ala Ala Leu Ala Phe Gln Asp Glu Met Cys Asp Ala Thr Thr
    130                 135                 140

GAT CAT GGC TTC GAC ATG GAG GAG ACG TTG GTG GAG GCT ATT TAC ACG   598
Asp His Gly Phe Asp Met Glu Glu Thr Leu Val Glu Ala Ile Tyr Thr
145                 150                 155                 160

GCG GAA CAG AGC GAA AAT GCG TTT TAT ATG CAC GAT GAG GCG ATG TTT   646
Ala Glu Gln Ser Glu Asn Ala Phe Tyr Met His Asp Glu Ala Met Phe
                165                 170                 175

GAG ATG CCG AGT TTG TTG GCT AAT ATG GCA GAA GGG ATG CTT TTG CCG   694
Glu Met Pro Ser Leu Leu Ala Asn Met Ala Glu Gly Met Leu Leu Pro
            180                 185                 190

CTT CCG TCC GTA CAG TGG AAT CAT AAT CAT GAA GTC GAC GGC GAT GAT   742
Leu Pro Ser Val Gln Trp Asn His Asn His Glu Val Asp Gly Asp Asp
        195                 200                 205

GAC GAC GTA TCG TTA TGG AGT TAT TAAAACTCAG ATTATTATTT CCATTTTTAG  796
Asp Asp Val Ser Leu Trp Ser Tyr
    210                 215

TACGATACTT TTTATTTTAT TATTATTTTT AGATCCTTTT TTAGAATGGA ATCTTCATTA 856

TGTTTGTAAA ACTGAGAAAC GAGTGTAAAT TAAATTGATT CAGTTTCAGT ATAAAAAAAA 916

AAAAAAAAAA AAAAAAA                                               933
```

(2) INFORMATION FOR SEQ ID NO.: 2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 216

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 2:

```
Met Asn Ser Phe Ser Ala Phe Ser Glu Met Phe Gly Ser Asp Tyr Glu
 1               5                  10                  15

Ser Ser Val Ser Ser Gly Gly Asp Tyr Ile Pro Thr Leu Ala Ser Ser
            20                  25                  30
```

```
Cys Pro Lys Lys Pro Ala Gly Arg Lys Lys Phe Arg Glu Thr Arg His
          35                  40                  45

Pro Ile Tyr Arg Gly Val Arg Arg Arg Asn Ser Gly Lys Trp Val Cys
     50                  55                  60

Glu Val Arg Glu Pro Asn Lys Lys Thr Arg Ile Trp Leu Gly Thr Phe
 65                  70                  75                  80

Gln Thr Ala Glu Met Ala Ala Arg Ala His Asp Val Ala Ala Leu Ala
                 85                  90                  95

Leu Arg Gly Arg Ser Ala Cys Leu Asn Phe Ala Asp Ser Ala Trp Arg
            100                 105                 110

Leu Arg Ile Pro Glu Ser Thr Cys Ala Lys Asp Ile Gln Lys Ala Ala
        115                 120                 125

Ala Glu Ala Ala Leu Ala Phe Gln Asp Glu Met Cys Asp Ala Thr Thr
    130                 135                 140

Asp His Gly Phe Asp Met Glu Glu Thr Leu Val Glu Ala Ile Tyr Thr
145                 150                 155                 160

Ala Glu Gln Ser Glu Asn Ala Phe Tyr Met His Asp Glu Ala Met Phe
                165                 170                 175

Glu Met Pro Ser Leu Leu Ala Asn Met Ala Glu Gly Met Leu Leu Pro
            180                 185                 190

Leu Pro Ser Val Gln Trp Asn His Asn His Glu Val Asp Gly Asp Asp
        195                 200                 205

Asp Asp Val Ser Leu Trp Ser Tyr
    210                 215
```

(2) INFORMATION FOR SEQ ID NO.: 3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1437

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE

(A) NAME/KEY: CDS

(B) LOCATION: (167)..(1171)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 3:

```
GCTGTCTGAT AAAAAGAAGA GGAAAACTCG AAAAAGCTAC ACACAAGAAG AAGAAGAAAA 60

GATACGAGCA AGAAGACTAA ACACGAAAGC GATTTATCAA CTCGAAGGAA GAGACTTTGA 120

TTTTCAAATT TCGTCCCCTA TAGATTGTGT TGTTTCTGGG AAGGAG ATG GCA GTT    175
                                                    Met Ala Val
                                                      1

TAT GAT CAG AGT GGA GAT AGA AAC AGA ACA CAA ATT GAT ACA TCG AGG    223
Tyr Asp Gln Ser Gly Asp Arg Asn Arg Thr Gln Ile Asp Thr Ser Arg
  5                  10                  15

AAA AGG AAA TCT AGA AGT AGA GGT GAC GGT ACT ACT GTG GCT GAG AGA    271
Lys Arg Lys Ser Arg Ser Arg Gly Asp Gly Thr Thr Val Ala Glu Arg
 20                  25                  30                  35

TTA AAG AGA TGG AAA GAG TAT AAC GAG ACC GTA GAA GAA GTT TCT ACC    319
Leu Lys Arg Trp Lys Glu Tyr Asn Glu Thr Val Glu Glu Val Ser Thr
                 40                  45                  50

AAG AAG AGG AAA GTA CCT GCG AAA GGG TCG AAG AAG GGT TGT ATG AAA    367
Lys Lys Arg Lys Val Pro Ala Lys Gly Ser Lys Lys Gly Cys Met Lys
             55                  60                  65

GGT AAA GGA GGA CCA GAG AAT AGC CGA TGT AGT TTC AGA GGA GTT AGG    415
Gly Lys Gly Gly Pro Glu Asn Ser Arg Cys Ser Phe Arg Gly Val Arg
         70                  75                  80

CAA AGG ATT TGG GGT AAA TGG GTT GCT GAG ATC AGA GAG CCT AAT CGA    463
Gln Arg Ile Trp Gly Lys Trp Val Ala Glu Ile Arg Glu Pro Asn Arg
     85                  90                  95

GGT AGC AGG CTT TGG CTT GGT ACT TTC CCT ACT GCT CAA GAA GCT GCT    511
Gly Ser Arg Leu Trp Leu Gly Thr Phe Pro Thr Ala Gln Glu Ala Ala
100                 105                 110                 115

TCT GCT TAT GAT GAG GCT GCT AAA GCT ATG TAT GGT CCT TTG GCT CGT    559
Ser Ala Tyr Asp Glu Ala Ala Lys Ala Met Tyr Gly Pro Leu Ala Arg
                120                 125                 130

CTT AAT TTC CCT CGG TCT GAT GCG TCT GAG GTT ACG AGT ACC TCA AGT    607
Leu Asn Phe Pro Arg Ser Asp Ala Ser Glu Val Thr Ser Thr Ser Ser
            135                 140                 145

CAG TCT GAG GTG TGT ACT GTT GAG ACT CCT GGT TGT GTT CAT GTG AAA    655
Gln Ser Glu Val Cys Thr Val Glu Thr Pro Gly Cys Val His Val Lys
        150                 155                 160

ACA GAG GAT CCA GAT TGT GAA TCT AAA CCC TTC TCC GGT GGA GTG GAG    703
Thr Glu Asp Pro Asp Cys Glu Ser Lys Pro Phe Ser Gly Gly Val Glu
    165                 170                 175

CCG ATG TAT TGT CTG GAG AAT GGT GCG GAA GAG ATG AAG AGA GGT GTT    751
Pro Met Tyr Cys Leu Glu Asn Gly Ala Glu Glu Met Lys Arg Gly Val
180                 185                 190                 195

AAA GCG GAT AAG CAT TGG CTG AGC GAG TTT GAA CAT AAC TAT TGG AGT    799
Lys Ala Asp Lys His Trp Leu Ser Glu Phe Glu His Asn Tyr Trp Ser
                200                 205                 210

GAT ATT CTG AAA GAG AAA GAG AAA CAG AAG GAG CAA GGG ATT GTA GAA    847
Asp Ile Leu Lys Glu Lys Glu Lys Gln Lys Glu Gln Gly Ile Val Glu
            215                 220                 225
```

```
ACC TGT CAG CAA CAA CAG CAG GAT TCG CTA TCT GTT GCA GAC TAT GGT   895
Thr Cys Gln Gln Gln Gln Asp Ser Leu Ser Val Ala Asp Tyr Gly
        230                 235                 240

TGG CCC AAT GAT GTG GAT CAG AGT CAC TTG GAT TCT TCA GAC ATG TTT   943
Trp Pro Asn Asp Val Asp Gln Ser His Leu Asp Ser Ser Asp Met Phe
    245                 250                 255

GAT GTC GAT GAG CTT CTA CGT GAC CTA AAT GGC GAC GAT GTG TTT GCA   991
Asp Val Asp Glu Leu Leu Arg Asp Leu Asn Gly Asp Asp Val Phe Ala
260                 265                 270                 275

GGC TTA AAT CAG GAC CGG TAC CCG GGG AAC AGT GTT GCC AAC GGT TCA  1039
Gly Leu Asn Gln Asp Arg Tyr Pro Gly Asn Ser Val Ala Asn Gly Ser
                280                 285                 290

TAC AGG CCC GAG AGT CAA CAA AGT GGT TTT GAT CCG CTA CAA AGC CTC  1087
Tyr Arg Pro Glu Ser Gln Gln Ser Gly Phe Asp Pro Leu Gln Ser Leu
            295                 300                 305

AAC TAC GGA ATA CCT CCG TTT CAG CTC GAG GGA AAG GAT GGT AAT GGA  1135
Asn Tyr Gly Ile Pro Pro Phe Gln Leu Glu Gly Lys Asp Gly Asn Gly
        310                 315                 320

TTC TTC GAC GAC TTG AGT TAC TTG GAT CTG GAG AAC TAAACAAAAC       1181
Phe Phe Asp Asp Leu Ser Tyr Leu Asp Leu Glu Asn
    325                 330                 335

AATATGAAGC TTTTTGGATT TGATATTTGC CTTAATCCCA CAACGACTGT TGATTCTCTA 1241

TCCGAGTTTT AGTGATATAG AGAACTACAG AACACGTTTT TTCTTGTTAT AAAGGTGAAC 1301

TGTATATATC GAAACAGTGA TATGACAATA GAGAAGACAA CTATAGTTTG TTAGTCTGCT 1361

TCTCTTAAGT TGTTCTTTAG ATATGTTTTA TGTTTTGTAA CAACAGGAAT GAATAATACA 1421

CACTTGTAAA AAAAAA                                                1437
```

(2) INFORMATION FOR SEQ ID NO.: 4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 335

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 4:

```
Met Ala Val Tyr Asp Gln Ser Gly Asp Arg Asn Arg Thr Gln Ile Asp
 1               5                  10                  15

Thr Ser Arg Lys Arg Lys Ser Arg Ser Arg Gly Asp Gly Thr Thr Val
            20                  25                  30

Ala Glu Arg Leu Lys Arg Trp Lys Glu Tyr Asn Glu Thr Val Glu Glu
        35                  40                  45

Val Ser Thr Lys Lys Arg Lys Val Pro Ala Lys Gly Ser Lys Lys Gly
    50                  55                  60

Cys Met Lys Gly Lys Gly Gly Pro Glu Asn Ser Arg Cys Ser Phe Arg
65                  70                  75                  80

Gly Val Arg Gln Arg Ile Trp Gly Lys Trp Val Ala Glu Ile Arg Glu
                85                  90                  95

Pro Asn Arg Gly Ser Arg Leu Trp Leu Gly Thr Phe Pro Thr Ala Gln
            100                 105                 110

Glu Ala Ala Ser Ala Tyr Asp Glu Ala Ala Lys Ala Met Tyr Gly Pro
        115                 120                 125

Leu Ala Arg Leu Asn Phe Pro Arg Ser Asp Ala Ser Glu Val Thr Ser
    130                 135                 140

Thr Ser Ser Gln Ser Glu Val Cys Thr Val Glu Thr Pro Gly Cys Val
145                 150                 155                 160

His Val Lys Thr Glu Asp Pro Asp Cys Glu Ser Lys Pro Phe Ser Gly
                165                 170                 175

Gly Val Glu Pro Met Tyr Cys Leu Glu Asn Gly Ala Glu Glu Met Lys
            180                 185                 190

Arg Gly Val Lys Ala Asp Lys His Trp Leu Ser Glu Phe Glu His Asn
        195                 200                 205

Tyr Trp Ser Asp Ile Leu Lys Glu Lys Glu Lys Gln Lys Glu Gln Gly
    210                 215                 220

Ile Val Glu Thr Cys Gln Gln Gln Gln Gln Asp Ser Leu Ser Val Ala
225                 230                 235                 240

Asp Tyr Gly Trp Pro Asn Asp Val Asp Gln Ser His Leu Asp Ser Ser
                245                 250                 255

Asp Met Phe Asp Val Asp Glu Leu Leu Arg Asp Leu Asn Gly Asp Asp
            260                 265                 270

Val Phe Ala Gly Leu Asn Gln Asp Arg Tyr Pro Gly Asn Ser Val Ala
        275                 280                 285

Asn Gly Ser Tyr Arg Pro Glu Ser Gln Gln Ser Gly Phe Asp Pro Leu
    290                 295                 300

Gln Ser Leu Asn Tyr Gly Ile Pro Pro Phe Gln Leu Glu Gly Lys Asp
305                 310                 315                 320

Gly Asn Gly Phe Phe Asp Asp Leu Ser Tyr Leu Asp Leu Glu Asn
                325                 330                 335
```

(2) INFORMATION FOR SEQ ID NO.: 5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 937

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE

(A) NAME/KEY: CDS

(B) LOCATION: (164)..(802)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 5:

```
CTTGAAAAAG AATCTACCTG AAAAGAAAAA AAAGAGAGAG AGATATAAAT AGCTTTACCA 60

AGACAGATAT ACTATCTTTT ATTAATCCAA AAAGACTGAG AACTCTAGTA ACTACGTACT 120

ACTTAAACCT TATCCAGTTT CTTGAAACAG AGTACTCTGA TCA ATG AAC TCA TTT   175
                                                Met Asn Ser Phe
                                                  1

TCA GCT TTT TCT GAA ATG TTT GGC TCC GAT TAC GAG CCT CAA GGC GGA   223
Ser Ala Phe Ser Glu Met Phe Gly Ser Asp Tyr Glu Pro Gln Gly Gly
  5                  10                  15                  20

GAT TAT TGT CCG ACG TTG GCC ACG AGT TGT CCG AAG AAA CCG GCG GGC   271
Asp Tyr Cys Pro Thr Leu Ala Thr Ser Cys Pro Lys Lys Pro Ala Gly
                 25                  30                  35

CGT AAG AAG TTT CGT GAG ACT CGT CAC CCA ATT TAC AGA GGA GTT CGT   319
Arg Lys Lys Phe Arg Glu Thr Arg His Pro Ile Tyr Arg Gly Val Arg
             40                  45                  50

CAA AGA AAC TCC GGT AAG TGG GTT TCT GAA GTG AGA GAG CCA AAC AAG   367
Gln Arg Asn Ser Gly Lys Trp Val Ser Glu Val Arg Glu Pro Asn Lys
         55                  60                  65

AAA ACC AGG ATT TGG CTC GGG ACT TTC CAA ACC GCT GAG ATG GCA GCT   415
Lys Thr Arg Ile Trp Leu Gly Thr Phe Gln Thr Ala Glu Met Ala Ala
     70                  75                  80

CGT GCT CAC GAC GTC GCT GCA TTA GCC CTC CGT GGC CGA TCA GCA TGT   463
Arg Ala His Asp Val Ala Ala Leu Ala Leu Arg Gly Arg Ser Ala Cys
 85                  90                  95                 100

CTC AAC TTC GCT GAC TCG GCT TGG CGG CTA CGA ATC CCG GAG TCA ACA   511
Leu Asn Phe Ala Asp Ser Ala Trp Arg Leu Arg Ile Pro Glu Ser Thr
                105                 110                 115
```

```
TGC GCC AAG GAT ATC CAA AAA GCG GCT GCT GAA GCG GCG TTG GCT TTT   559
Cys Ala Lys Asp Ile Gln Lys Ala Ala Ala Glu Ala Ala Leu Ala Phe
            120                 125                 130

CAA GAT GAG ACG TGT GAT ACG ACG ACC ACG AAT CAT GGC CTG GAC ATG   607
Gln Asp Glu Thr Cys Asp Thr Thr Thr Thr Asn His Gly Leu Asp Met
        135                 140                 145

GAG GAG ACG ATG GTG GAA GCT ATT TAT ACA CCG GAA CAG AGC GAA GGT   655
Glu Glu Thr Met Val Glu Ala Ile Tyr Thr Pro Glu Gln Ser Glu Gly
    150                 155                 160

GCG TTT TAT ATG GAT GAG GAG ACA ATG TTT GGG ATG CCG ACT TTG TTG   703
Ala Phe Tyr Met Asp Glu Glu Thr Met Phe Gly Met Pro Thr Leu Leu
165                 170                 175                 180

GAT AAT ATG GCT GAA GGC ATG CTT TTA CCG CCG CCG TCT GTT CAA TGG   751
Asp Asn Met Ala Glu Gly Met Leu Leu Pro Pro Pro Ser Val Gln Trp
                185                 190                 195

AAT CAT AAT TAT GAC GGC GAA GGA GAT GGT GAC GTG TCG CTT TGG AGT   799
Asn His Asn Tyr Asp Gly Glu Gly Asp Gly Asp Val Ser Leu Trp Ser
            200                 205                 210

TAC TAATATTCGA TAGTCGTTTC CATTTTTGTA CTATAGTTTG AAAATATTCT        852
Tyr

AGTTCCTTTT TTTAGAATGG TTCCTTCATT TTATTTTATT TTATTGTTGT AGAAACGAGT 912

GGAAAATAAT TCAATACAAA AAAAA                                       937
```

(2) INFORMATION FOR SEQ ID NO.: 6:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 213

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 6:

```
Met Asn Ser Phe Ser Ala Phe Ser Glu Met Phe Gly Ser Asp Tyr Glu
  1               5                  10                  15

Pro Gln Gly Gly Asp Tyr Cys Pro Thr Leu Ala Thr Ser Cys Pro Lys
            20                  25                  30

Lys Pro Ala Gly Arg Lys Lys Phe Arg Glu Thr Arg His Pro Ile Tyr
        35                  40                  45
```

```
Arg Gly Val Arg Gln Arg Asn Ser Gly Lys Trp Val Ser Glu Val Arg
     50                  55                  60

Glu Pro Asn Lys Lys Thr Arg Ile Trp Leu Gly Thr Phe Gln Thr Ala
 65                  70                  75                  80

Glu Met Ala Ala Arg Ala His Asp Val Ala Ala Leu Ala Leu Arg Gly
                 85                  90                  95

Arg Ser Ala Cys Leu Asn Phe Ala Asp Ser Ala Trp Arg Leu Arg Ile
            100                 105                 110

Pro Glu Ser Thr Cys Ala Lys Asp Ile Gln Lys Ala Ala Ala Glu Ala
        115                 120                 125

Ala Leu Ala Phe Gln Asp Glu Thr Cys Asp Thr Thr Thr Thr Asn His
    130                 135                 140

Gly Leu Asp Met Glu Glu Thr Met Val Glu Ala Ile Tyr Thr Pro Glu
145                 150                 155                 160

Gln Ser Glu Gly Ala Phe Tyr Met Asp Glu Glu Thr Met Phe Gly Met
                165                 170                 175

Pro Thr Leu Leu Asp Asn Met Ala Glu Gly Met Leu Leu Pro Pro Pro
            180                 185                 190

Ser Val Gln Trp Asn His Asn Tyr Asp Gly Glu Gly Asp Gly Asp Val
        195                 200                 205

Ser Leu Trp Ser Tyr
    210
```

(2) INFORMATION FOR SEQ ID NO.: 7:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 944

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE

(A) NAME/KEY: CDS

(B) LOCATION: (135)..(782)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 7:

```
CCTGAATTAG AAAAGAAAGA TAGATAGAGA AATAAATATT TTATCATACC ATACAAAAAA 60

AGACAGAGAT CTTCTACTTA CTCTACTCTC ATAAACCTTA TCCAGTTTCT TGAAACAGAG 120
```

```
TACTCTTCTG ATCA ATG AAC TCA TTT TCT GCC TTT TCT GAA ATG TTT GGC   170
                Met Asn Ser Phe Ser Ala Phe Ser Glu Met Phe Gly
                  1               5                  10

TCC GAT TAC GAG TCT CCG GTT TCC TCA GGC GGT GAT TAC AGT CCG AAG   218
Ser Asp Tyr Glu Ser Pro Val Ser Ser Gly Gly Asp Tyr Ser Pro Lys
        15                  20                  25

CTT GCC ACG AGC TGC CCC AAG AAA CCA GCG GGA AGG AAG AAG TTT CGT   266
Leu Ala Thr Ser Cys Pro Lys Lys Pro Ala Gly Arg Lys Lys Phe Arg
    30                  35                  40

GAG ACT CGT CAC CCA ATT TAC AGA GGA GTT CGT CAA AGA AAC TCC GGT   314
Glu Thr Arg His Pro Ile Tyr Arg Gly Val Arg Gln Arg Asn Ser Gly
 45                  50                  55                  60

AAG TGG GTG TGT GAG TTG AGA GAG CCA AAC AAG AAA ACG AGG ATT TGG   362
Lys Trp Val Cys Glu Leu Arg Glu Pro Asn Lys Lys Thr Arg Ile Trp
                65                  70                  75

CTC GGG ACT TTC CAA ACC GCT GAG ATG GCA GCT CGT GCT CAC GAC GTC   410
Leu Gly Thr Phe Gln Thr Ala Glu Met Ala Ala Arg Ala His Asp Val
            80                  85                  90

GCC GCC ATA GCT CTC CGT GGC AGA TCT GCC TGT CTC AAT TTC GCT GAC   458
Ala Ala Ile Ala Leu Arg Gly Arg Ser Ala Cys Leu Asn Phe Ala Asp
        95                  100                 105

TCG GCT TGG CGG CTA CGA ATC CCG GAA TCA ACC TGT GCC AAG GAA ATC   506
Ser Ala Trp Arg Leu Arg Ile Pro Glu Ser Thr Cys Ala Lys Glu Ile
    110                 115                 120

CAA AAG GCG GCG GCT GAA GCC GCG TTG AAT TTT CAA GAT GAG ATG TGT   554
Gln Lys Ala Ala Ala Glu Ala Ala Leu Asn Phe Gln Asp Glu Met Cys
125                 130                 135                 140

CAT ATG ACG ACG GAT GCT CAT GGT CTT GAC ATG GAG GAG ACC TTG GTG   602
His Met Thr Thr Asp Ala His Gly Leu Asp Met Glu Glu Thr Leu Val
                145                 150                 155

GAG GCT ATT TAT ACG CCG GAA CAG AGC CAA GAT GCG TTT TAT ATG GAT   650
Glu Ala Ile Tyr Thr Pro Glu Gln Ser Gln Asp Ala Phe Tyr Met Asp
            160                 165                 170

GAA GAG GCG ATG TTG GGG ATG TCT AGT TTG TTG GAT AAC ATG GCC GAA   698
Glu Glu Ala Met Leu Gly Met Ser Ser Leu Leu Asp Asn Met Ala Glu
        175                 180                 185

GGG ATG CTT TTA CCG TCG CCG TCG GTT CAA TGG AAC TAT AAT TTT GAT   746
Gly Met Leu Leu Pro Ser Pro Ser Val Gln Trp Asn Tyr Asn Phe Asp
    190                 195                 200

GTC GAG GGA GAT GAT GAC GTG TCC TTA TGG AGC TAT TAAAATTCGA        792
Val Glu Gly Asp Asp Asp Val Ser Leu Trp Ser Tyr
205                 210                 215

TTTTTATTTC CATTTTTGGT ATTATAGCTT TTTATACATT TGATCCTTTT TTAGAATGGA 852

TCTTCTTCTT TTTTTGGTTG TGAGAAACGA ATGTAAATGG TAAAAGTTGT TGTCAAATGC 912

AAATGTTTTT GAGTGCAGAA TATATAATCT TT                               944
```

(2) INFORMATION FOR SEQ ID NO.: 8:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 216

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 8:

```
Met Asn Ser Phe Ser Ala Phe Ser Glu Met Phe Gly Ser Asp Tyr Glu
 1               5                  10                  15

Ser Pro Val Ser Ser Gly Gly Asp Tyr Ser Pro Lys Leu Ala Thr Ser
            20                  25                  30

Cys Pro Lys Lys Pro Ala Gly Arg Lys Lys Phe Arg Glu Thr Arg His
        35                  40                  45

Pro Ile Tyr Arg Gly Val Arg Gln Arg Asn Ser Gly Lys Trp Val Cys
    50                  55                  60

Glu Leu Arg Glu Pro Asn Lys Lys Thr Arg Ile Trp Leu Gly Thr Phe
65                  70                  75                  80

Gln Thr Ala Glu Met Ala Ala Arg Ala His Asp Val Ala Ala Ile Ala
                85                  90                  95

Leu Arg Gly Arg Ser Ala Cys Leu Asn Phe Ala Asp Ser Ala Trp Arg
            100                 105                 110

Leu Arg Ile Pro Glu Ser Thr Cys Ala Lys Glu Ile Gln Lys Ala Ala
        115                 120                 125

Ala Glu Ala Ala Leu Asn Phe Gln Asp Glu Met Cys His Met Thr Thr
    130                 135                 140

Asp Ala His Gly Leu Asp Met Glu Glu Thr Leu Val Glu Ala Ile Tyr
145                 150                 155                 160

Thr Pro Glu Gln Ser Gln Asp Ala Phe Tyr Met Asp Glu Glu Ala Met
                165                 170                 175

Leu Gly Met Ser Ser Leu Leu Asp Asn Met Ala Glu Gly Met Leu Leu
            180                 185                 190

Pro Ser Pro Ser Val Gln Trp Asn Tyr Asn Phe Asp Val Glu Gly Asp
        195                 200                 205

Asp Asp Val Ser Leu Trp Ser Tyr
    210                 215
```

(2) INFORMATION FOR SEQ ID NO.: 9:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1513

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(ix) FEATURE

(A) NAME/KEY: CDS

(B) LOCATION: (183)..(1172)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 9:

```
GAGACGCTAG AAAGAACGCG AAAGCTTGCG AAGAAGATTT GCTTTTGATC GACTTAACAC 60

GAACAACAAA CAACATCTGC GTGATAAAGA AGAGATTTTT GCCTAAATAA AGAAGAGATT 120

CGACTCTAAT CCTGGAGTTA TCATTCACGA TAGATTCTTA GATTGCGACT ATAAAGAAGA 180

AG ATG GCT GTA TAT GAA CAA ACC GGA ACC GAG CAG CCG AAG AAA AGG     227
   Met Ala Val Tyr Glu Gln Thr Gly Thr Glu Gln Pro Lys Lys Arg
     1               5                  10                  15

AAA TCT AGG GCT CGA GCA GGT GGT TTA ACG GTG GCT GAT AGG CTA AAG   275
Lys Ser Arg Ala Arg Ala Gly Gly Leu Thr Val Ala Asp Arg Leu Lys
                20                  25                  30

AAG TGG AAA GAG TAC AAC GAG ATT GTT GAA GCT TCG GCT GTT AAA GAA   323
Lys Trp Lys Glu Tyr Asn Glu Ile Val Glu Ala Ser Ala Val Lys Glu
            35                  40                  45

GGA GAG AAA CCG AAA CGC AAA GTT CCT GCG AAA GGG TCG AAG AAA GGT   371
Gly Glu Lys Pro Lys Arg Lys Val Pro Ala Lys Gly Ser Lys Lys Gly
        50                  55                  60

TGT ATG AAG GGT AAA GGA GGA CCA GAT AAT TCT CAC TGT AGT TTT AGA   419
Cys Met Lys Gly Lys Gly Gly Pro Asp Asn Ser His Cys Ser Phe Arg
    65                  70                  75

GGA GTT AGA CAA AGG ATT TGG GGT AAA TGG GTT GCA GAG ATT CGA GAA   467
Gly Val Arg Gln Arg Ile Trp Gly Lys Trp Val Ala Glu Ile Arg Glu
80                  85                  90                  95

CCG AAA ATA GGA ACT AGA CTT TGG CTT GGT ACT TTT CCT ACC GCG GAA   515
Pro Lys Ile Gly Thr Arg Leu Trp Leu Gly Thr Phe Pro Thr Ala Glu
                100                 105                 110
```

```
AAA GCT GCT TCC GCT TAT GAT GAA GCG GCT ACC GCT ATG TAC GGT TCA   563
Lys Ala Ala Ser Ala Tyr Asp Glu Ala Ala Thr Ala Met Tyr Gly Ser
            115                 120                 125

TTG GCT CGT CTT AAC TTC CCT CAG TCT GTT GGG TCT GAG TTT ACT AGT   611
Leu Ala Arg Leu Asn Phe Pro Gln Ser Val Gly Ser Glu Phe Thr Ser
        130                 135                 140

ACG TCT AGT CAA TCT GAG GTG TGT ACG GTT GAA AAT AAG GCG GTT GTT   659
Thr Ser Ser Gln Ser Glu Val Cys Thr Val Glu Asn Lys Ala Val Val
145                 150                 155

TGT GGT GAT GTT TGT GTG AAG CAT GAA GAT ACT GAT TGT GAA TCT AAT   707
Cys Gly Asp Val Cys Val Lys His Glu Asp Thr Asp Cys Glu Ser Asn
160                 165                 170                 175

CCA TTT AGT CAG ATT TTA GAT GTT AGA GAA GAG TCT TGT GGA ACC AGG   755
Pro Phe Ser Gln Ile Leu Asp Val Arg Glu Glu Ser Cys Gly Thr Arg
                180                 185                 190

CCG GAC AGT TGC ACG GTT GGA CAT CAA GAT ATG AAT TCT TCG CTG AAT   803
Pro Asp Ser Cys Thr Val Gly His Gln Asp Met Asn Ser Ser Leu Asn
            195                 200                 205

TAC GAT TTG CTG TTA GAG TTT GAG CAG CAG TAT TGG GGC CAA GTT TTG   851
Tyr Asp Leu Leu Leu Glu Phe Glu Gln Gln Tyr Trp Gly Gln Val Leu
        210                 215                 220

CAG GAG AAA GAG AAA CCG AAG CAG GAA GAA GAG GAG ATA CAG CAA CAG   899
Gln Glu Lys Glu Lys Pro Lys Gln Glu Glu Glu Glu Ile Gln Gln Gln
225                 230                 235

CAA CAG GAA CAG CAA CAG CAA CAG CTG CAA CCG GAT TTG CTT ACT GTT   947
Gln Gln Glu Gln Gln Gln Gln Gln Leu Gln Pro Asp Leu Leu Thr Val
240                 245                 250                 255

GCA GAT TAC GGT TGG CCT TGG TCT AAT GAT ATT GTA AAT GAT CAG ACT   995
Ala Asp Tyr Gly Trp Pro Trp Ser Asn Asp Ile Val Asn Asp Gln Thr
                260                 265                 270

TCT TGG GAT CCT AAT GAG TGC TTT GAT ATT AAT GAA CTC CTT GGA GAT  1043
Ser Trp Asp Pro Asn Glu Cys Phe Asp Ile Asn Glu Leu Leu Gly Asp
            275                 280                 285

TTG AAT GAA CCT GGT CCC CAT CAG AGC CAA GAC CAA AAC CAC GTA AAT  1091
Leu Asn Glu Pro Gly Pro His Gln Ser Gln Asp Gln Asn His Val Asn
        290                 295                 300

TCT GGT AGT TAT GAT TTG CAT CCG CTT CAT CTC GAG CCA CAC GAT GGT  1139
Ser Gly Ser Tyr Asp Leu His Pro Leu His Leu Glu Pro His Asp Gly
305                 310                 315

CAC GAG TTC AAT GGT TTG AGT TCT CTG GAT ATT TGAGAGTTCT GAGGCAATGG 1192
His Glu Phe Asn Gly Leu Ser Ser Leu Asp Ile
320                 325                 330

TCCTACAAGA CTACAACATA ATCTTTGGAT TGATCATAGG AGAAACAAGA AATAGGTGTT 1252

AATGATCTGA TTCACAATGA AAAAATATTT AATAACTCTA TAGTTTTTGT TCTTTCCTTG 1312

GATCATGAAC TGTTGCTTCT CATCTATTGA GTTAATATAG CGAATAGCAG AGTTTCTCTC 1372

TTTCTTCTCT TTGTAGAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAYH SAKMABGCAR 1432
```

SRCSDVSNAA NNTRNATNAR SARCHCNTRR AGRCTRASCN CSRCASWASH TSKBABARAK 1492

AANTAMAYSA KMASRNGNGA C 1513

(2) INFORMATION FOR SEQ ID NO.: 10:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 330

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: polypeptide

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 10:

```
Met Ala Val Tyr Glu Gln Thr Gly Thr Glu Gln Pro Lys Lys Arg Lys
 1               5                  10                  15

Ser Arg Ala Arg Ala Gly Gly Leu Thr Val Ala Asp Arg Leu Lys Lys
            20                  25                  30

Trp Lys Glu Tyr Asn Glu Ile Val Glu Ala Ser Ala Val Lys Glu Gly
        35                  40                  45

Glu Lys Pro Lys Arg Lys Val Pro Ala Lys Gly Ser Lys Lys Gly Cys
    50                  55                  60

Met Lys Gly Lys Gly Gly Pro Asp Asn Ser His Cys Ser Phe Arg Gly
65                  70                  75                  80

Val Arg Gln Arg Ile Trp Gly Lys Trp Val Ala Glu Ile Arg Glu Pro
                85                  90                  95

Lys Ile Gly Thr Arg Leu Trp Leu Gly Thr Phe Pro Thr Ala Glu Lys
            100                 105                 110

Ala Ala Ser Ala Tyr Asp Glu Ala Ala Thr Ala Met Tyr Gly Ser Leu
        115                 120                 125

Ala Arg Leu Asn Phe Pro Gln Ser Val Gly Ser Glu Phe Thr Ser Thr
    130                 135                 140

Ser Ser Gln Ser Glu Val Cys Thr Val Glu Asn Lys Ala Val Val Cys
145                 150                 155                 160

Gly Asp Val Cys Val Lys His Glu Asp Thr Asp Cys Glu Ser Asn Pro
                165                 170                 175

Phe Ser Gln Ile Leu Asp Val Arg Glu Glu Ser Cys Gly Thr Arg Pro
            180                 185                 190
```

Asp Ser Cys Thr Val Gly His Gln Asp Met Asn Ser Ser Leu Asn Tyr
195 200 205

Asp Leu Leu Leu Glu Phe Glu Gln Gln Tyr Trp Gly Gln Val Leu Gln
210 215 220

Glu Lys Glu Lys Pro Lys Gln Glu Glu Glu Glu Ile Gln Gln Gln Gln
225 230 235 240

Gln Glu Gln Gln Gln Gln Gln Leu Gln Pro Asp Leu Leu Thr Val Ala
245 250 255

Asp Tyr Gly Trp Pro Trp Ser Asn Asp Ile Val Asn Asp Gln Thr Ser
260 265 270

Trp Asp Pro Asn Glu Cys Phe Asp Ile Asn Glu Leu Leu Gly Asp Leu
275 280 285

Asn Glu Pro Gly Pro His Gln Ser Gln Asp Gln Asn His Val Asn Ser
290 295 300

Gly Ser Tyr Asp Leu His Pro Leu His Leu Glu Pro His Asp Gly His
305 310 315 320

Glu Phe Asn Gly Leu Ser Ser Leu Asp Ile
325 330

(2) INFORMATION FOR SEQ ID NO.: 11:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Designed oligonucleotide based on the promoter region of rd29A gene and having HindIII site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 11:

AAGCTTAAGC TTACATCAGT TTGAAAGAAA 30

(2) INFORMATION FOR SEQ ID NO.: 12:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 31

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Designed oligonucleotide based on the promoter region of rd29A gene and having HindIII site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 12:

AAGCTTAAGC TTGCTTTTTG GAACTCATGT C 31

(2) INFORMATION FOR SEQ ID NO.: 13:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 32

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Designed oligonucleotide based on DREB1A gene and having BamHI site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 13:

AAGCTTAAGC TTGCCATAGA TGCAATTCAA TC 32

(2) INFORMATION FOR SEQ ID NO.: 14:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 36

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Designed oligonucleotide based on DREB1A gene and having BamHI site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 14:

AAGCTTAAGC TTTTCCAAAG ATTTTTTTCT TTCCAA 36

(2) INFORMATION FOR SEQ ID NO.: 15:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Designed oligonucleotide based on the promoter region of rd29A gene and having HindIII site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 15:

GGATCCGGAT CCATGAACTC ATTTTCTGCT 30

(2) INFORMATION FOR SEQ ID NO.: 16:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 32

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Designed oligonucleotide based on the promoter region of rd29A gene and having HindIII site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 16:

GGATCCGGAT CCTTAATAAC TCCATAACGA TA 32

(2) INFORMATION FOR SEQ ID NO.: 17:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 941

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 17:

GCCATAGATG CAATTCAATC AAACTGAAAT TTCTGCAAGA ATCTCAAACA CGGAGATCTC 60

AAAGTTTGAA AGAAAATTTA TTTCTTCGAC TCAAAACAAA CTTACGAAAT TTAGGTAGAA 120

CTTATATACA TTATATTGTA ATTTTTTGTA ACAAAATGTT TTTATTATTA TTATAGAATT 180

TTACTGGTTA AATTAAAAAT GAATAGAAAA GGTGAATTAA GAGGAGAGAG GAGGTAAACA 240

TTTTCTTCTA TTTTTTCATA TTTTCAGGAT AAATTATTGT AAAAGTTTAC AAGATTTCCA 300

TTTGACTAGT GTAAATGAGG AATATTCTCT AGTAAGATCA TTATTTCATC TACTTCTTTT 360

ATCTTCTACC AGTAGAGGAA TAAACAATAT TTAGCTCCTT TGTAAATACA AATTAATTTT 420

CCTTCTTGAC ATCATTCAAT TTTAATTTTA CGTATAAAAT AAAAGATCAT ACCTATTAGA 480

ACGATTAAGG AGAAATACAA TTCGAATGAG AAGGATGTGC CGTTTGTTAT AATAAACAGC 540

CACACGACGT AAACGTAAAA TGACCACATG ATGGGCCAAT AGACATGGAC CGACTACTAA 600

TAATAGTAAG TTACATTTTA GGATGGAATA AATATCATAC CGACATCAGT TTTGAAAGAA 660

AAGGGAAAAA AAGAAAAAAT AAATAAAAGA TATACTACCG ACATGAGTTC CAAAAAGCAA 720

AAAAAAAGAT CAAGCCGACA CAGACACGCG TAGAGAGCAA AATGACTTTG ACGTCACACC 780

ACGAAAACAG ACGCTTCATA CGTGTCCCTT TATCTCTCTC AGTCTCTCTA TAAACTTAGT 840

GAGACCCTCC TCTGTTTTAC TCACAAATAT GCAAACTAGA AAACAATCAT CAGGAATAAA 900

GGGTTTGATT ACTTCTATTG GAAAGAAAAA AATCTTTGGA A 941

(2) INFORMATION FOR SEQ ID NO.: 18:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 71

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Arabidopsis thaliana

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 18:

CAGTTTGAAA GAAAAGGGAA AAAAAGAAAA AATAAATAAA AGATATACTA CCGACATGAG 60

TTCCAAAAAG C 71

(2) INFORMATION FOR SEQ ID NO.: 19:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 71

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Oligonucleotide having a partially mutated sequence within the DRE region.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 19:

CAGTTTGAAA GAAAAGGGAA AAAAAGAAAA AATAAATAAA AGATATATTT TCGACATGAG 60

TTCCAAAAAG C 71

(2) INFORMATION FOR SEQ ID NO.: 20:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 71

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Oligonucleotide having a partially mutated sequence within the DRE region.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 20:

CAGTTTGAAA GAAAAGGGAA AAAAAGAAAA AATAAATAAA AGATATACTA CTTTTATGAG 60

TTCCAAAAAG C 71

(2) INFORMATION FOR SEQ ID NO.: 21:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 71

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Oligonucleotide having a partially mutated sequence within the DRE region.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 21:

CAGTTTGAAA GAAAAGGGAA AAAAAGAAAA AATAAATAAA AGATATACTA CCGACAAAAG 60

TTCCAAAAAG C 71

(2) INFORMATION FOR SEQ ID NO.: 22:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 71

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Oligonucleotide having a partially mutated sequence outside the DRE region.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 22:

CAGTTTGAAA GAAAAGGGAA AAAAAGAAAA AATAAATAAA AGATATACTA CCGACATGAT 60

CAACAAAAAG C 71

(2) INFORMATION FOR SEQ ID NO.: 23:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 71

(B) TYPE: nucleic acid

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Artificial Sequence

(ix) FEATURE

(C) OTHER INFORMATION: Oligonucleotide having a partially mutated sequence outside the DRE region.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO.: 23:

CAGTTTGAAA GAAAAGGGAA AAAAAGAAAA AATAAATAAA AGATATACTA CCGACATGAG 60

TTCGGTTAAG C 71

What is claimed is:

1. A transgenic plant containing a gene in which a DNA encoding the following protein (a) or (b) is ligated downstream of a stress responsive promoter:

(a) a protein consisting of the amino acid sequence as shown in SEQ I D NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10;

(b) a protein which consists of the amino acid sequence having deletion, substitution or addition of at least one amino acid in the amino acid sequence as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 10 and which regulates the transcription of genes located downstream of a stress responsive element.

2. A transgenic plant containing a gene in which the following DNA (c) or (d) is ligated downstream of a stress responsive promoter:

(c) a DNA consisting of the nucleotide sequence as shown in SEQ ID NO : 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9;

(d) a DNA which hybridizes with the DNA consisting of the nucleotide sequence as shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9 under stringent conditions and which codes for a protein that regulates the transcription of genes located downstream of a stress responsive element.

3. The transgenic plant of claim 1 or 2, wherein the stress is dehydration stress, low temperature stress or salt stress.

4. The transgenic plant of any one of claims 1 to 3, wherein the stress responsive promoter is at least one selected from the group

consisting of rd29A gene promoter, rd29B gene promoter, rd17 gene promoter, rd22 gene promoter, DREB1A gene promoter, cor6.6 gene promoter, cor15a gene promoter, erd1 gene promoter and kin1 gene promoter.

FIG. 1

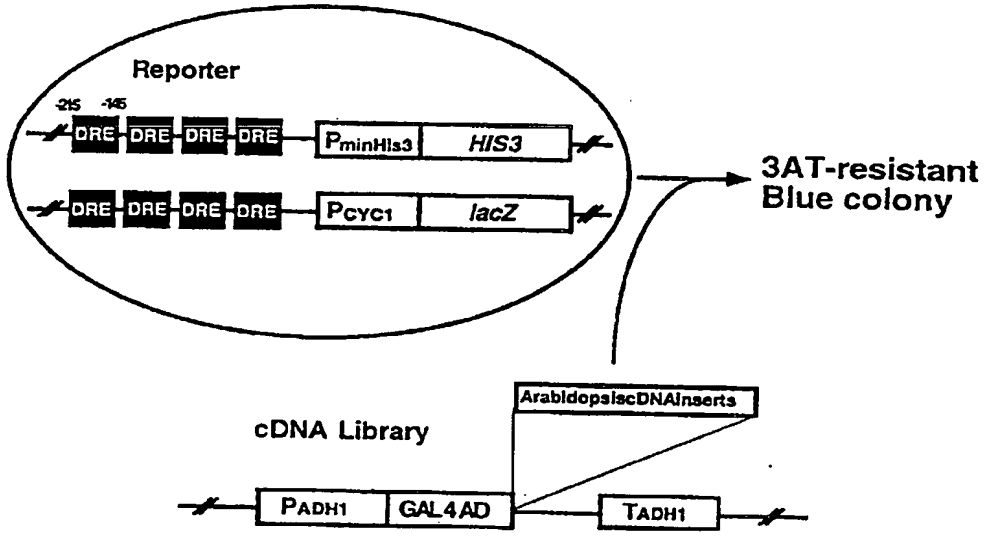

Reporter
-215
-145
DRE
DRE
DRE
DRE
PminHis3
HIS3
DRE
DRE
DRE
DRE
PCYC1
lacZ
3AT-resistant
Blue colony
cDNA Library
ArabidopsiscDNAinserts
PADH1
GAL4AD
TADH1

FIG. 2

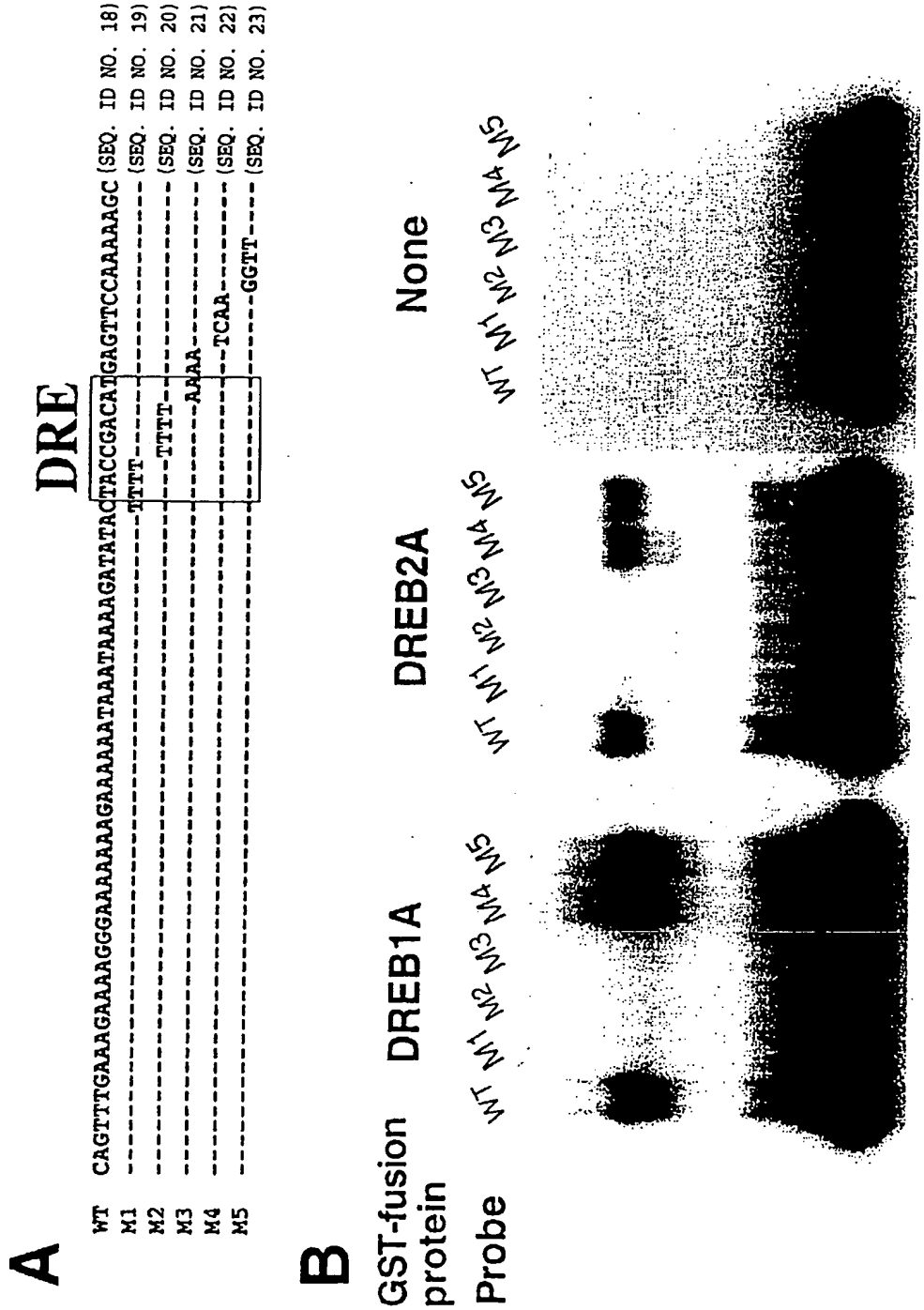

A
DRE
WT CAGTTTGAAAGAAAAGGGAAAAAAGAAAAAATAAATAAAGATATACTACCGACATGAGTTCCAAAAAGC (SEQ. ID NO. 18)
M1 ----------TTT----------- (SEQ. ID NO. 19)
M2 ----------TTT----------- (SEQ. ID NO. 20)
M3 ----------AAAA---------- (SEQ. ID NO. 21)
M4 ----------TCAA---------- (SEQ. ID NO. 22)
M5 ----------GGTT---------- (SEQ. ID NO. 23)
B
GST-fusion protein
DREB1A
DREB2A
None
Probe
WT M1 M2 M3 M4 M5
WT M1 M2 M3 M4 M5
WT M1 M2 M3 M4 M5

FIG. 3

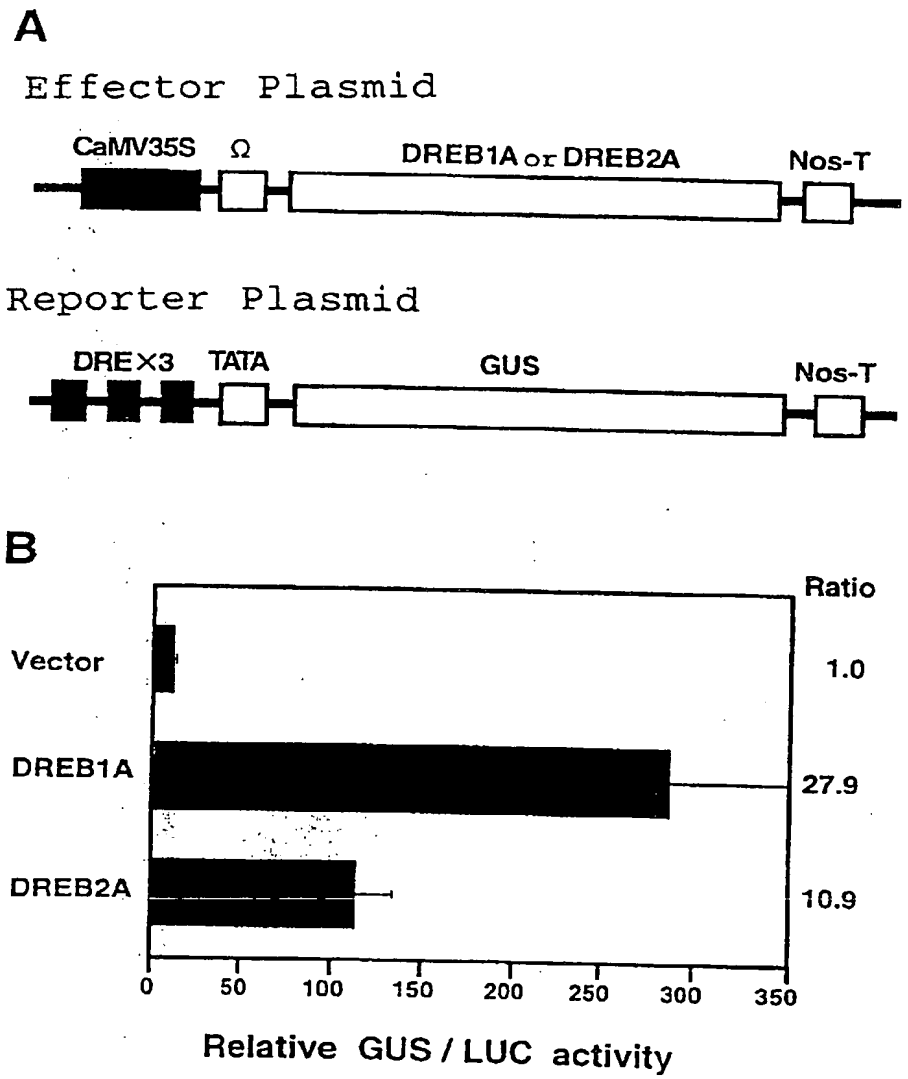

A
Effector Plasmid
CaMV35S
Ω
DREB1A or DREB2A
Nos-T
Reporter Plasmid
DRE×3
TATA
GUS
Nos-T
B
Ratio
Vector
1.0
DREB1A
27.9
DREB2A
10.9
0
50
100
150
200
250
300
350
Relative GUS / LUC activity

FIG. 4

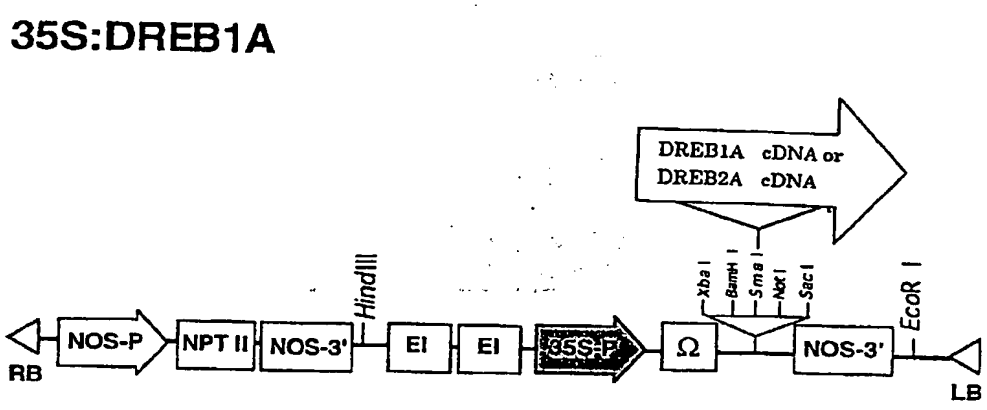

35S:DREB1A
DREB1A cDNA or
DREB2A cDNA
Xba I
BamH I
Sma I
Not I
Sac I
HindIII
EcoR I
NOS-P
NPT II
NOS-3'
EI
EI
35S-P
Ω
NOS-3'
RB
LB

FIG. 5

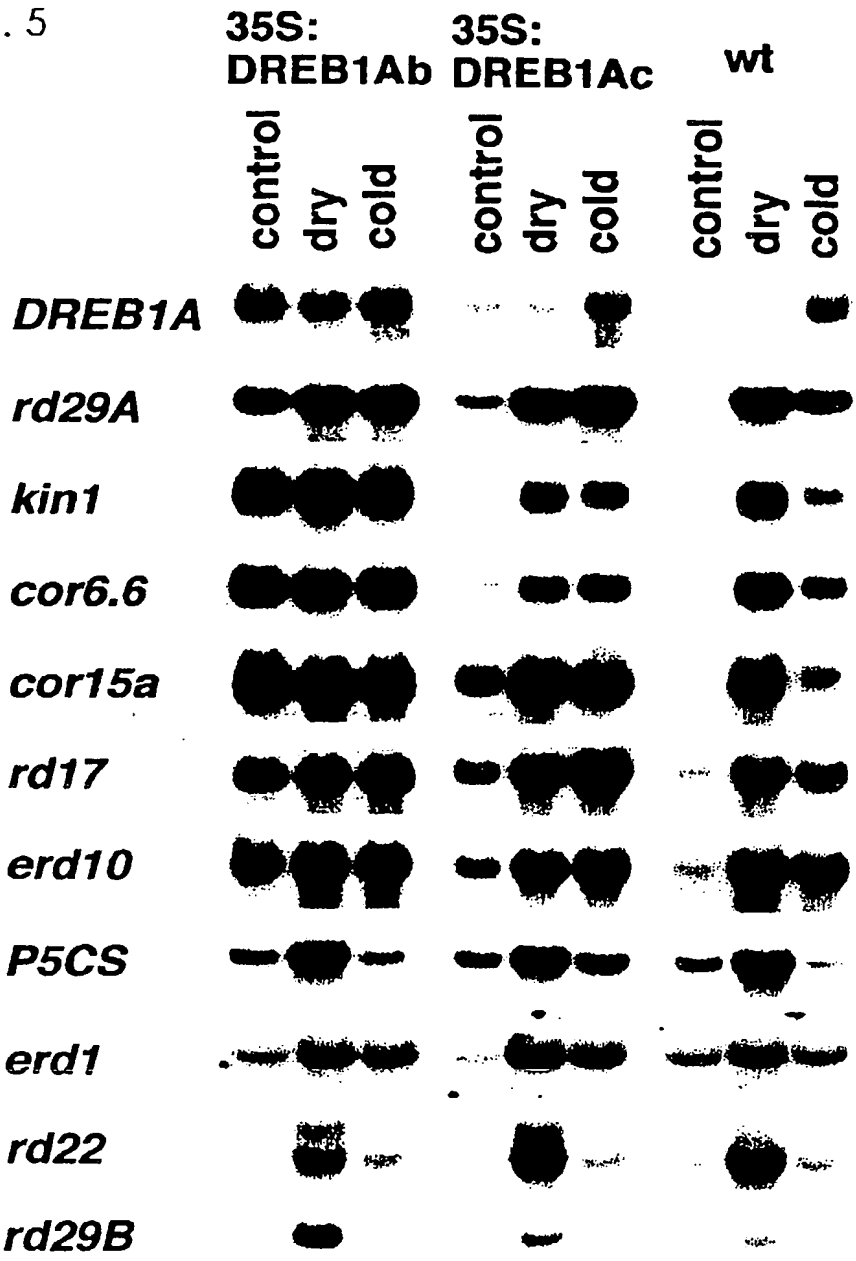

35S: DREB1Ab
35S: DREB1Ac
wt
control
dry
cold
control
dry
cold
control
dry
cold
DREB1A
rd29A
kin1
cor6.6
cor15a
rd17
erd10
P5CS
erd1
rd22
rd29B

FIG. 6

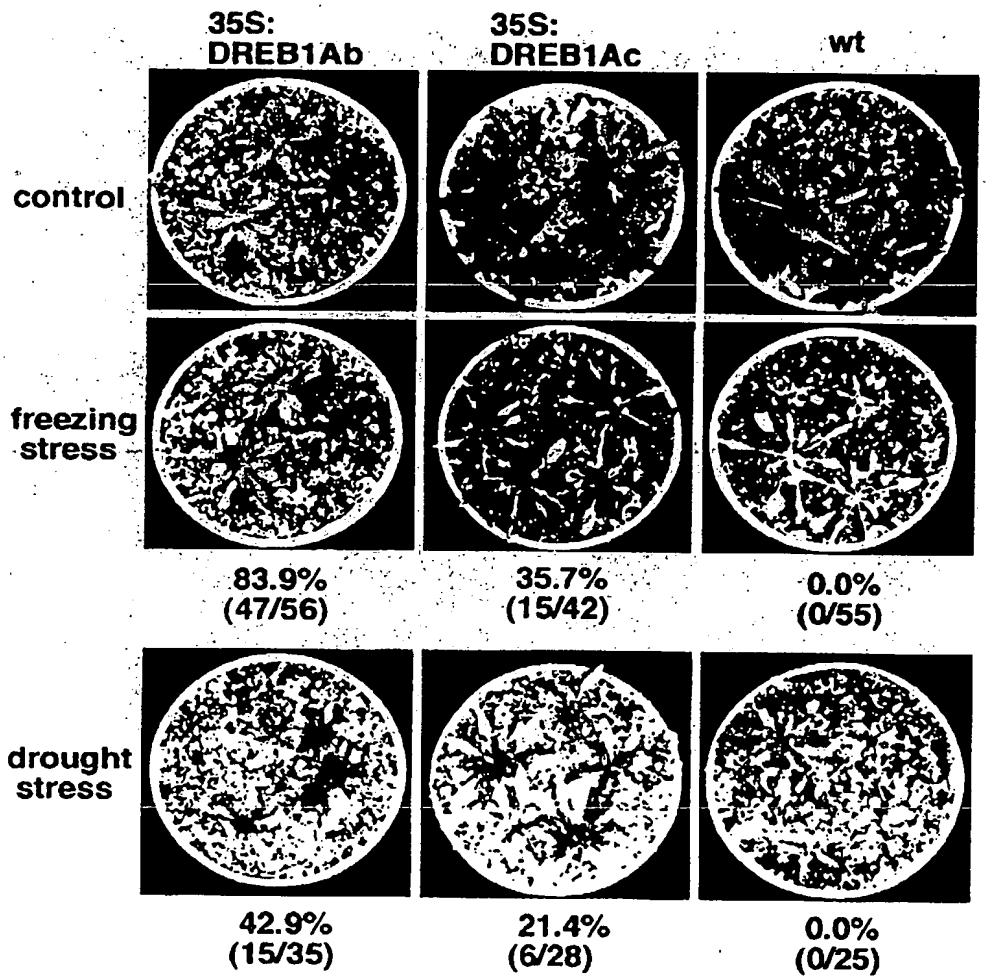

35S:
DREB1Ab
35S:
DREB1Ac
wt
control
freezing
stress
83.9%
(47/56)
35.7%
(15/42)
0.0%
(0/55)
drought
stress
42.9%
(15/35)
21.4%
(6/28)
0.0%
(0/25)

FIG. 7

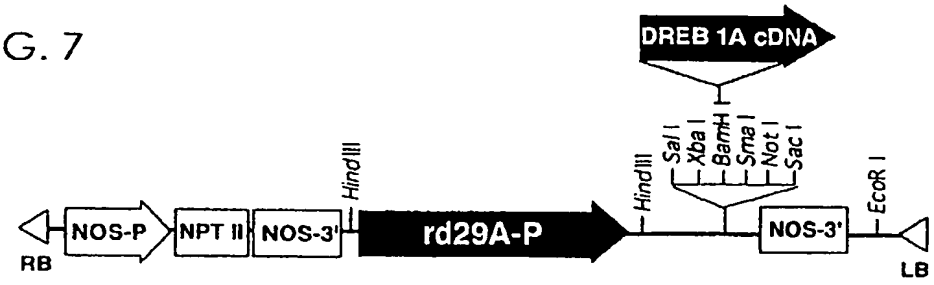

DREB 1A cDNA
Sal I
Xba I
BamH
Sma I
Not I
Sac I
HindIII
HindIII
EcoR I
NOS-P
NPT II
NOS-3'
rd29A-P
NOS-3'
RB
LB

FIG. 8

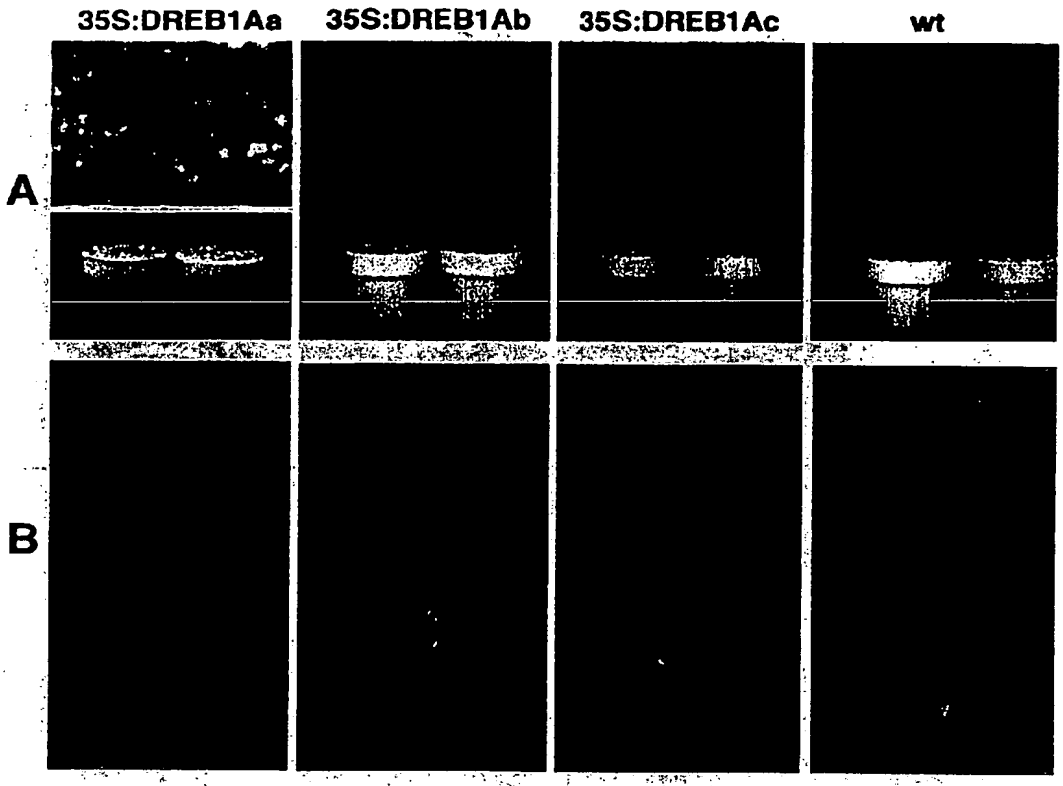


FIG. 9

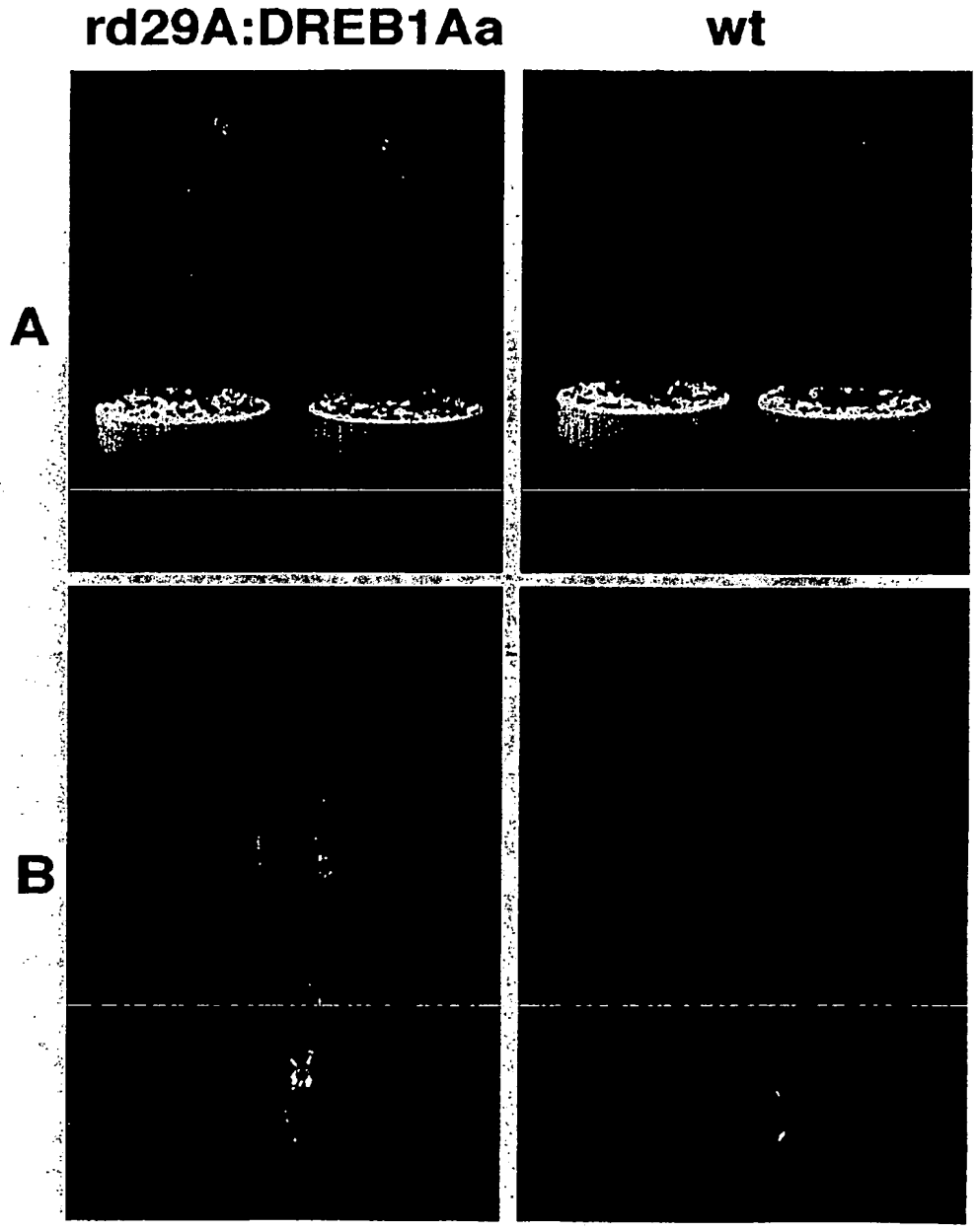
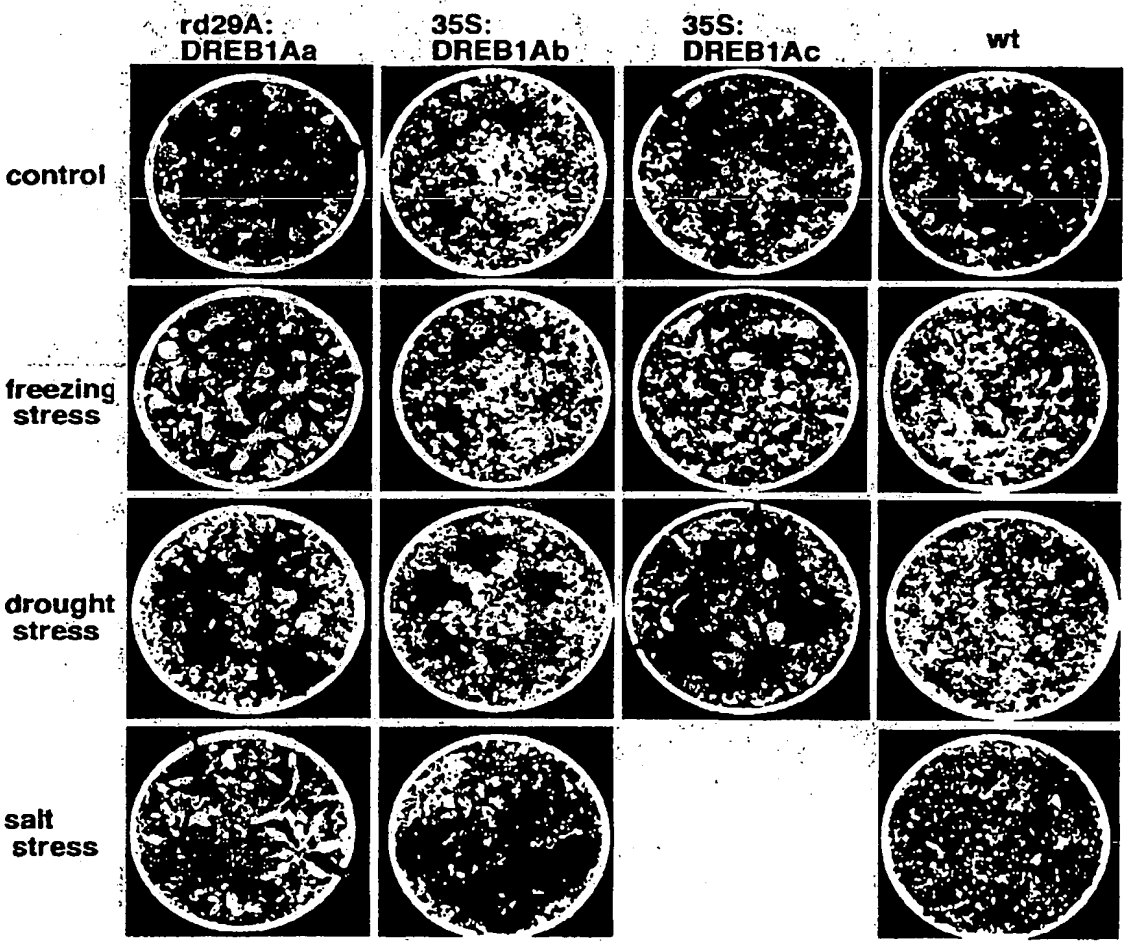


FIG. 10


rd29A:
DREB1Aa
35S:
DREB1Ab
35S:
DREB1Ac
wt
control
freezing
stress
drought
stress
salt
stress